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BACTERIOLOGICAL STUDIES OF COMMERCIAL SHELLFISH OPERATION ON THE GULF COAST

M. W. Presnell, et al

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

1961

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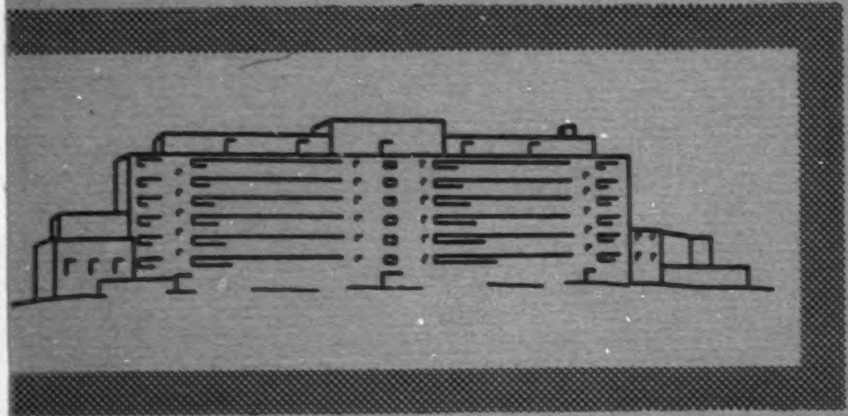

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BACTERIOLOGICAL STUDIES OF COMMERCIAL SHELLFISH OPERATIONS ON THE GULF COAST

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U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Division of Environmental Engineering and Food Protection

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

1961

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BACTERIOLOGICAL STUDIES OF COMMERCIAL SHELLFISH OPERATIONS ON THE GULF COAST

PART I. OBSERVATIONS IN OYSTER AREAS

SUMMARY

A field study was conducted in four approved growing areas on the Gulf Coast to determine oyster/water coliform relationships and the compliance of shellfish growing waters and oysters as harvested with the recommended bacteriological limits of the Public Health Service Manual of Recommended Practice for the Sanitary Control of the Shellfish Industry.

In all areas, the coliform density of the oysters was consistently higher than that of the water, but the extent of bacterial accumulation varied in any given area and from area to area. (m) Run-off which resulted in higher bacterial content of the water and lower salinity, was the most important factor associated with the variations in bacterial accumulation. The coliform content of the oysters usually increased during conditions of low salinity, reflecting the changes in the bacterial density of the water, but the increases were not proportionate, probably because of the inhibition of feeding rate by lower salinity. Oyster feeding was more consistent and at a higher rate under higher salinity conditions.

Three of the four areas complied with water coliform criteria recommended for approved growing areas. One of the three areas showed a very high degree of compliance. The remaining two areas complied with the recommended median MPN of 70 and only slightly exceeded the 10 percent limit recommended for MPN 230. The fourth area did not comply with the recommended water criteria probably because this area was subjected to abnormally high run-off during the period of study.

Regardless of the degree to which waters complied with recommended bacteriological criteria, oysters from all areas failed to comply with the coliform limits recommended for eastern oysters at the point of harvesting or shucking in the North or Middle Atlantic area. Results of the study show that the currently recommended coliform MPN level of 2400 per 100 grams of meat is a more reasonable standard for Gulf Coast oysters, as harvested, than the MPN limit of 230 formerly applied to oysters from the area. However, the use of a dual coliform standard tends to cast doubt on the validity of the entire coliform group as an index of pollution. It is suggested that perhaps *E. coli* would be a more meaningful indicator organism.

The potential of bottom deposits as contributors to the coliform content of water and oysters was demonstrated. Bottom materials consistently showed higher MPN's than overlying water. During periods of turbulence resuspension could occur, thereby effecting an increase in the coliform content of the water and possibly a concurrent increase in the oyster coliform density.

INTRODUCTION

The rate of bacterial accumulation by the eastern oyster, *Crassostrea virginica*, at controlled water coliform levels has been determined in laboratory experiments conducted near Pensacola, Florida.[1] These studies were undertaken primarily to obtain basic information for the establishment of acceptable bacteriological levels for shellfish growing waters and shellfish as harvested from marine estuaries of the Gulf of Mexico. In addition to the determination of the bacterial relationships between the water and the oyster, these experiments demonstrated seasonal variations in the rate of bacterial accumulation, with the highest rates occurring when commercial harvesting is at a high level, in the late fall and early spring.

It was recognized that controlled laboratory experiments could not simulate completely the varied conditions occurring in natural environments. Furthermore, the laboratory experiments were conducted in only one producing area. Therefore, in an attempt to correlate laboratory findings with observations in natural environments and to determine possible variations in oyster/water coliform relationships from area to area it was considered advisable to study these relationships in several commercial oyster growing areas.

This report describes the results of bacteriological examinations of water, oyster, and bottom-deposit samples collected simultaneously in four representative commercial shellfish producing areas on the Gulf Coast. The general objectives were to determine:

1. The oyster/water coliform relationships in natural environments.
2. The degree to which Gulf Coast shellfish and shellfish waters complied with current recommended bacteriological criteria.
3. The potential of bottom deposits as contributors to the coliform content of water and oysters.

MATERIALS AND METHODS

Description of Areas

The four sites selected for study are all important commercial producing areas on the Gulf Coast and are approved by state regulatory authorities for the growing and direct marketing of oysters. The areas are shown in Figures 1, 2, 3, and 4. For identification purposes they are designated by the letters A, B, M, and P. The hydrography of all areas is typical of growing waters on the Gulf Coast. All four areas are relatively small estuarine bodies of water with average depths of 6 to 8 feet. Tidal amplitude is low, with a diurnal range of about 1.5 feet between mean low and high water. Relatively large rivers empty into the areas. During periods of heavy rainfall there is considerable fresh water dilution, resulting in significant reductions in salinity.

Because of the nature of the study, sampling stations necessarily were located over productive oyster reefs. When possible, however, sampling stations were located to give representative sampling of an area. In most instances two samples of water and two of oysters were collected from a station on each sampling date. The sampling was so spaced that a time interval of 2 to 4 hours elapsed between collections, to allow the collection of samples under a wider variety of tidal conditions. Some stations were so remote that only single daily samplings were possible. At stations where the nature of the bottom permitted, samples of bottom deposits were collected once on each sampling date.

Collection of Samples

Samples of water for bacteriological examination were collected in sterile bottles within a few inches of the bottom. Samples from this depth are considered most desirable in bacteriological surveys of growing areas because they represent water immediately in contact with oysters. All water samples were collected by means of a sampler that permitted rapid, efficient collection at any desired depth. This sampler is illustrated in Figure 5.

A minimum of 12 oysters were tonged or dredged from each station. The shellfish were placed in sterile, impervious containers and stored at temperatures between 0 and 10°C until examined.

Bottom deposit samples consisted of the pooled material from at least three corings. Corings were made by means of the sampler illustrated in Figure 6. In preparation for coring, the tip was removed from the sampler and a sterile plexiglass sampling tube inserted and secured in position by replacement of the tip. The sampler was attached to a wooden handle, lowered to the bottom, and inserted to a proper depth. Experience with the sampler soon enabled the operator to determine the pressure of insertion required to obtain a core that retained itself but did not exceed the length of the tube. The sampling tube was removed and placed in a vertical position in a metal cylindrical container. After a settling period of approximately 10 minutes, a plunger was inserted into the bottom of the tube and the excess water forced out. Approximately 10 centimeters of water were left at the top to avoid loss of the more flocculent portions of the bottom deposits. This upper layer, being more flocculent, would be most liable to resuspension during periods of turbulence. A sterile sample jar was then put in place, and the 10 centimeters of water and approximately 10 centimeters of bottom deposits were transferred to the jar. This resulted in approximately 100 ml of sample.

When sampling at a station was completed, the sampler and sampling tube were thoroughly scrubbed and immersed for 10 minutes in a solution containing approximately 1000 ppm available chlorine. The chlorine solution was rinsed from

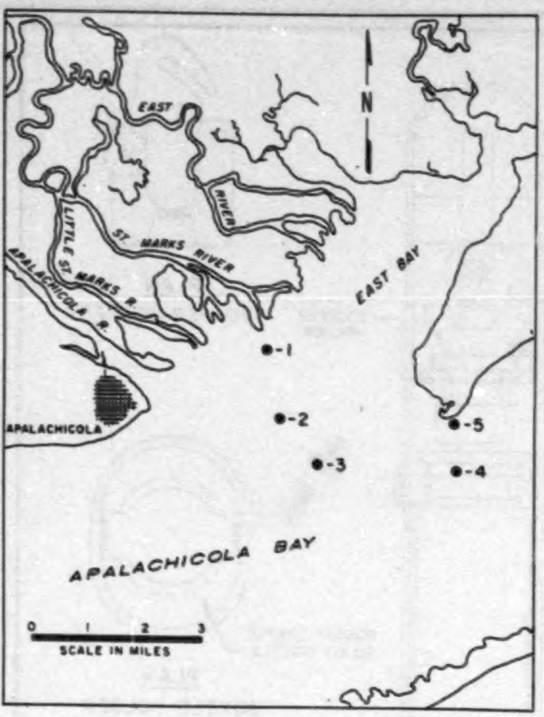
N
EAST
RIVER
LITTLE ST. MARKS R.
ST. MARKS RIVER
APALACHICOLA R.
EAST BAY
APALACHICOLA
●-1
●-2
●-3
●-4
●-5
APALACHICOLA BAY
0 1 2 3
SCALE IN MILES

Figure 1. Area A

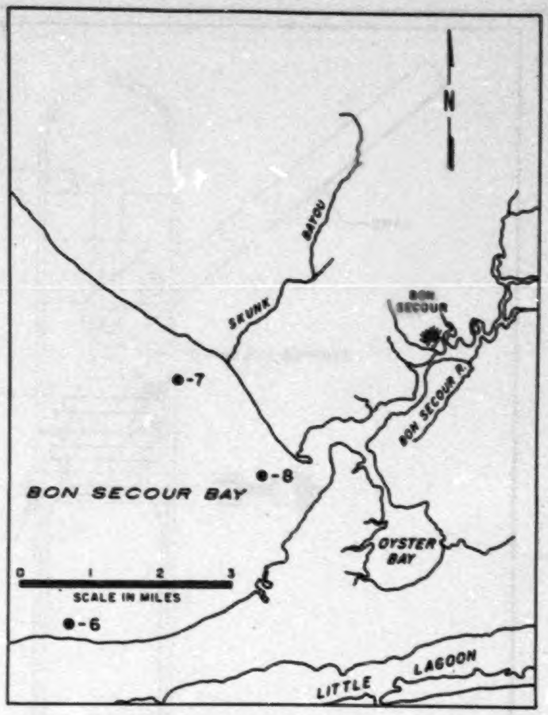
N
BAYOU
SKUNK
BON SECOUR
BON SECOUR R.
●-7
●-8
BON SECOUR BAY
OYSTER BAY
0 1 2 3
SCALE IN MILES
●-6
LITTLE LAGOON

Figure 2. Area B

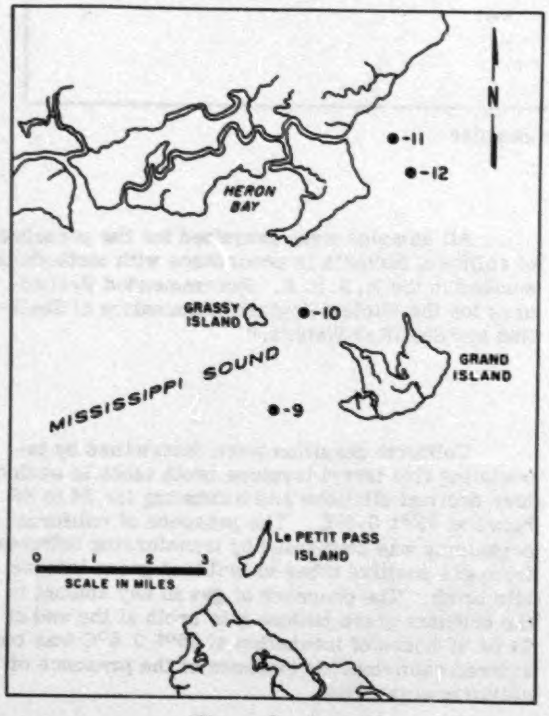
N
●-11
●-12
HERON BAY
GRASSY ISLAND
●-10
GRAND ISLAND
MISSISSIPPI SOUND
●-9
Le PETIT PASS ISLAND
0 1 2 3
SCALE IN MILES

Figure 3. Area M

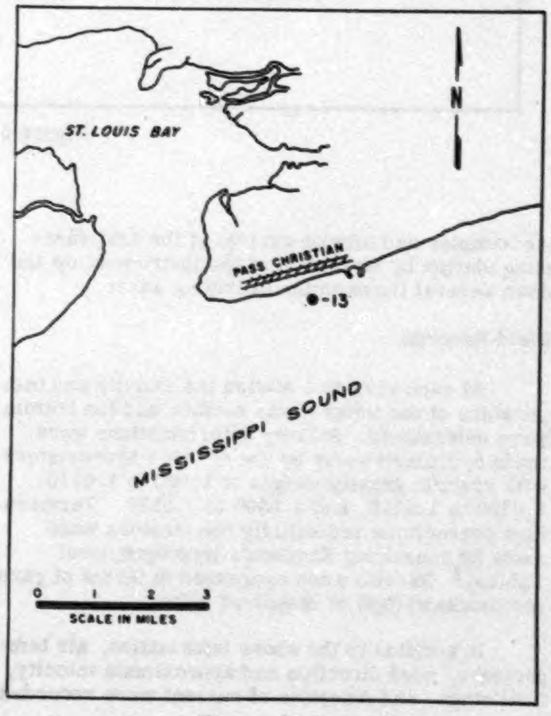
N
ST. LOUIS BAY
PASS CHRISTIAN
●-13
MISSISSIPPI SOUND
0 1 2 3
SCALE IN MILES

Figure 4. Area P

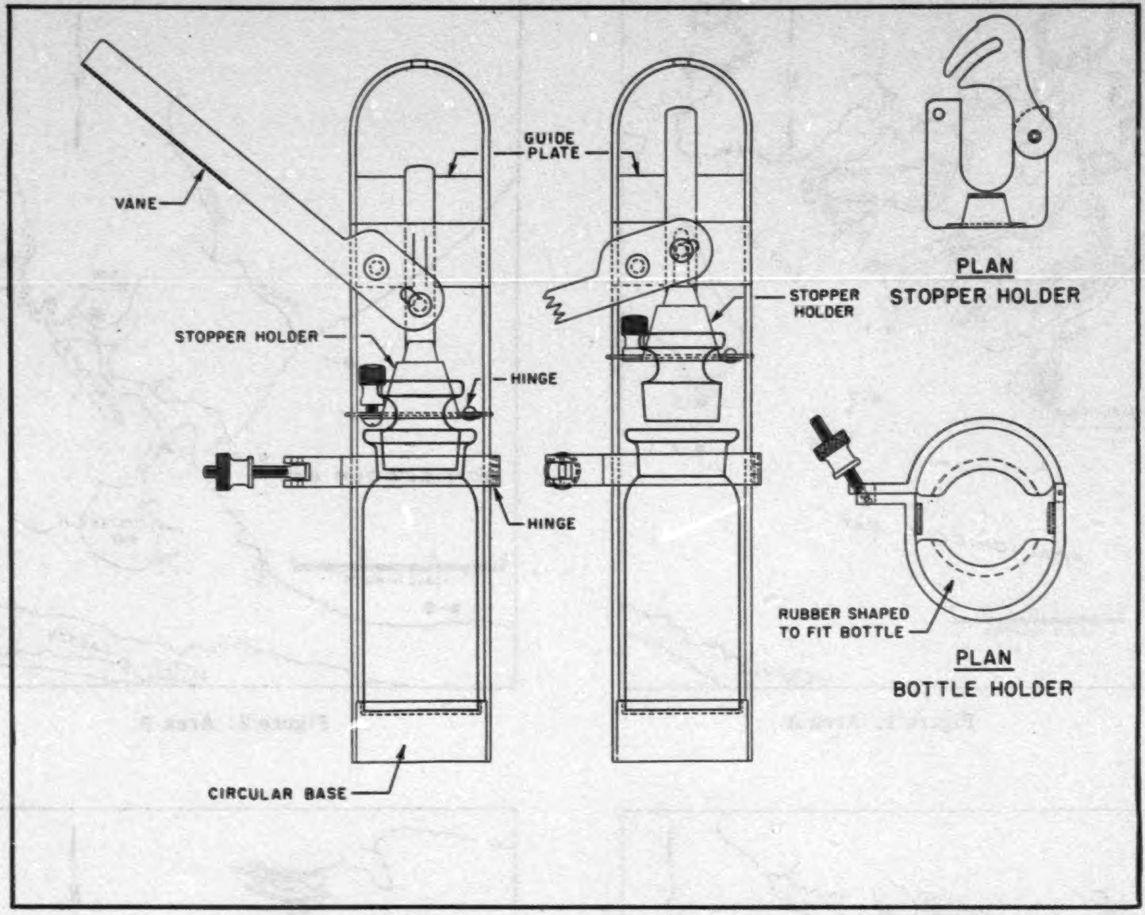

Figure 5. Water sampler

the sampler and tube on arrival at the next sampling station by movement of the instrument up and down several times in the overlying water.

Field Records

At each visit to a station the salinity and temperature of the water at the surface and the bottom were determined. Salinity determinations were made hydrometrically by use of three hydrometers with specific gravity ranges of 1.000 to 1.0110, 1.0100 to 1.0210, and 1.0200 to 1.0310. Temperature corrections and salinity conversions were made by consulting Knudsen's Hydrographical Tables.[2] Results were expressed in terms of parts per thousand (ppt) of dissolved salts.

In addition to the above information, air temperature, wind direction and approximate velocity, tidal stage, and direction of current were recorded.

All samples were examined for the presence of coliform bacteria in accordance with methods described in the A.P.H.A. Recommended Procedures for the Bacteriological Examination of Shellfish and Shellfish Waters.[3]

Coliform densities were determined by inoculating five lauryl tryptose broth tubes in each of four decimal dilutions and incubating for 24 to 48 hours at $35^\circ \pm 0.5^\circ$C. The presence of coliform organisms was confirmed by transferring cultures from gas positive tubes to brilliant green lactose bile broth. The presence of gas in any amount in the brilliant green lactose bile broth at the end of 24 or 48 hours of incubation at $35^\circ \pm 0.5^\circ$C was considered confirmatory evidence of the presence of coliform organisms.

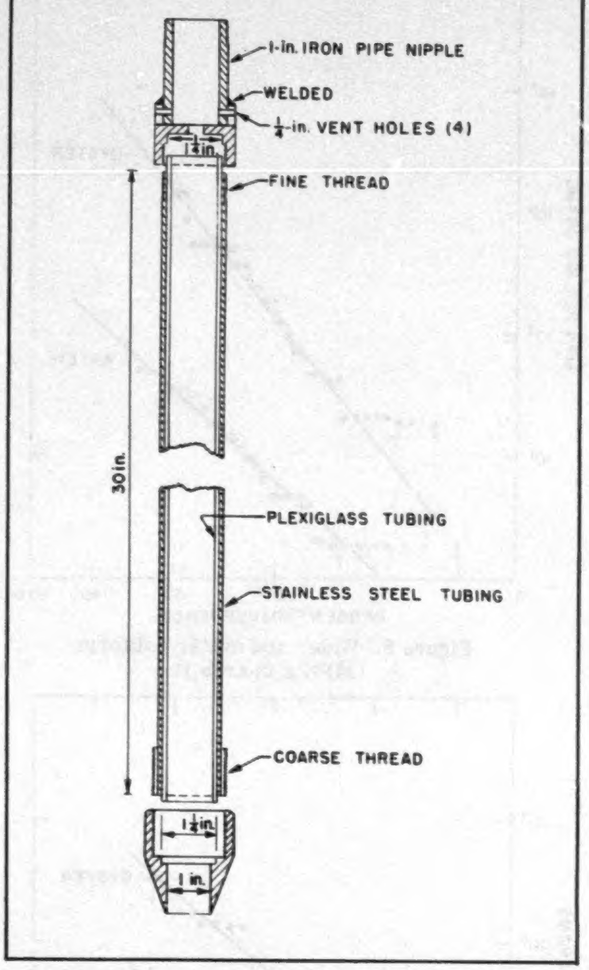

Figure 6. Bottom stratification sampler

Plate counts were made on all oyster samples to collect baseline data for use in the studies of bacterial changes in oysters during harvesting and processing (described in part II of this report).

All samples of water and bottom deposits were inoculated into presumptive broth on board the boat immediately after collection. Oyster samples were prepared and inoculated within 2 hours or less of the time of collection. The coliform density of water and oyster samples was expressed in the Most Probable Number (MPN) of organisms per 100 ml and 100 grams, respectively. The coliform density of bottom deposits was expressed in terms of the MPN per 100 ml of settleable solids. Settleable solids were determined volumetrically by measuring the quantity of solids in 100 ml of sample that had settled after 24 hours in a graduated cylinder. Preliminary trials demonstrated that optimum settling had occurred within this period and that the supernatant had clarified. Results computed on the basis of settleable solids were considered most representative of coliform densities of that portion of bottom materials that might, through resuspension, influence the coliform content of water and oysters.

RESULTS

In area A, 15 sampling tours were conducted over a period extending from June 1956 to October 1957. In area B, 19 sampling tours were conducted from February 1956 to August 1957. During a period extending from November 1957 to April 1958, a total of eight tours were conducted in areas M and P. A complete tabulation of the data is included in the appendix.

For the purpose of summarization, plots of the data were prepared on logarithmic probability paper, in accordance with the method described by Velz. [4] Plots of water and oyster coliform MPN's obtained from areas A, B, M, and P are presented in Figures 7 through 10. All of the data show a normal pattern of distribution, as indicated by the proximity of the plotted values to a straight line fitted to the mass of the data.

Oyster MPN's were consistently higher than water MPN's in all areas, but oyster/water coliform relationships varied within an area and from area to area. An estimate of the degree of uptake of bacteria by the oysters can be obtained from the spread between the water and the oyster lines. This spread varies from area to area, indicating variation between areas in the degree of bacterial accumulation. For example, comparison of Figures 7 and 8 shows that the lines are farther apart in plots from area B, indicating that the oyster coliform content, relative to water coliform density, was higher in area B than in area A.

The coliform content of water and oyster samples from the four areas differed in both geometric mean (50 percentile level) and range (10-90 percentile level). Area B showed the lowest mean water and oyster MPN's, followed by areas A, P, and M. The lowest range of water MPN's also occurred in B, followed by P, M, and A. The lowest range of oyster MPN's was obtained in P, followed by areas A, B, and M.

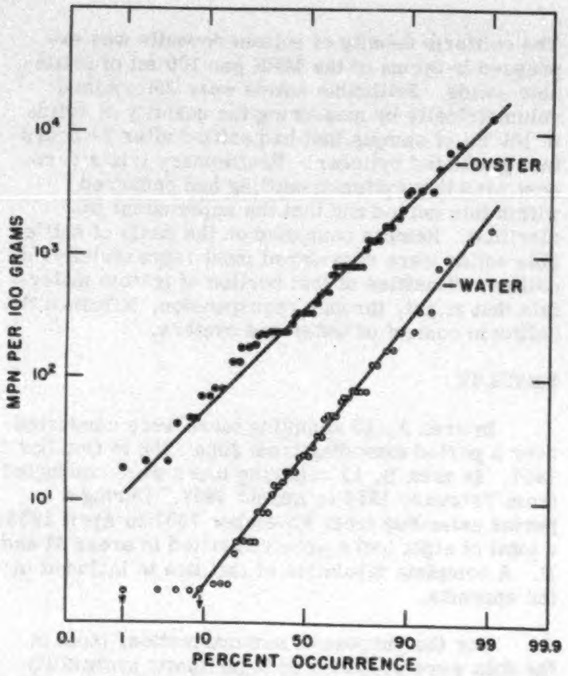

Figure 7. Water and oyster coliform MPN's in area A

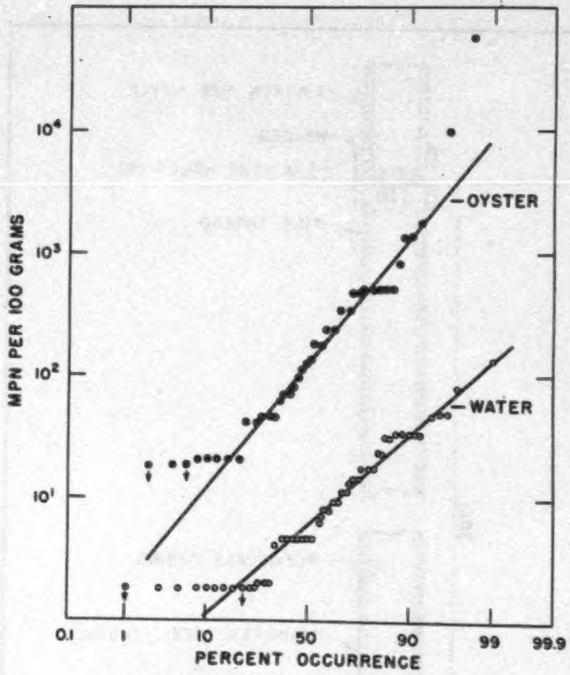

Figure 8. Water and oyster coliform MPN's in area B

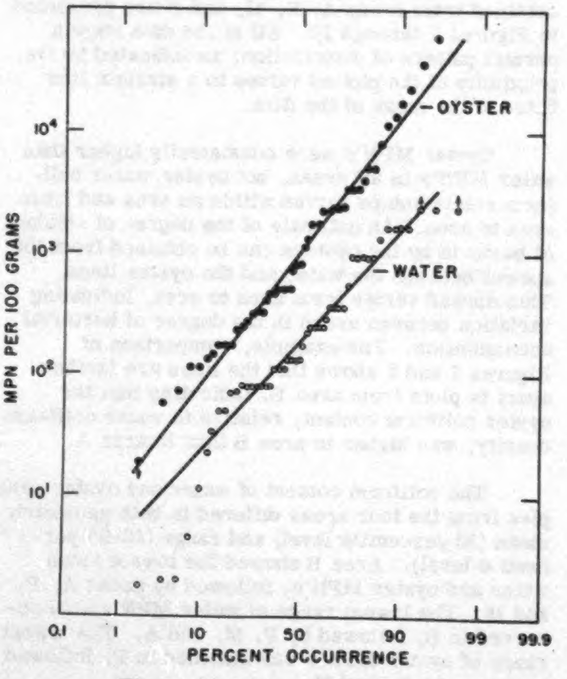

Figure 9. Water and oyster coliform MPN's in area M

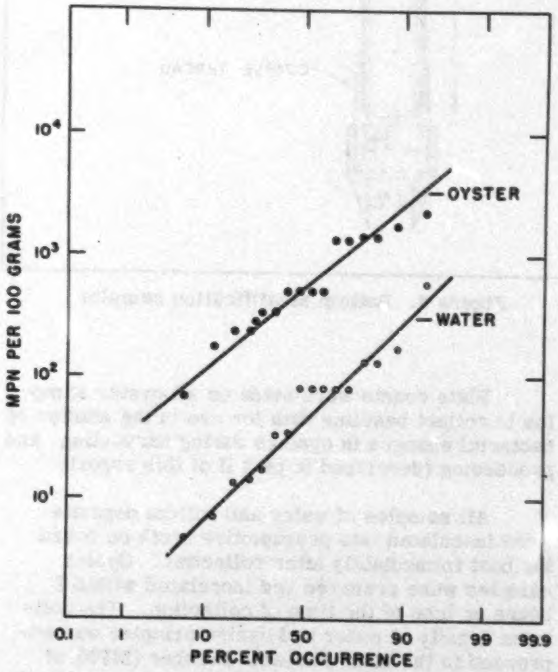

Figure 10. Water and oyster coliform MPN's in area P

TABLE 1. WATER AND OYSTER COLIFORM MPN'S IN FOUR APPROVED SHELLFISH GROWING AREAS

Probability percentile[a]	Area A		Area B		Area M		Area P	
	Water	Oyster	Water	Oyster	Water	Oyster	Water	Oyster
10	2.1	54	1.1	12	23	80	6.6	110
50	25	380	5.9	130	190	1,000	43	540
90	300	2,700	33	1,300	1,500	13,000	280	2,600
No. of Samples	64	83	76	51	51	51	16	16

[a] Derived from plots on log probability paper.

Water and oyster MPN's at the 10, 50, and 90 percentile levels have been taken from the above plots and are presented in Table 1.

In order to determine the cause of variations in oyster/water coliform relationships and in water and oyster coliform densities, an attempt was made to correlate these variations with water temperature, tidal changes, fresh run-off as indicated by salinity fluctuations, and season. In all areas variations were closely associated with run-off.

The average annual rainfall throughout the region of study is approximately 60 inches. Consequently, the salinity of the relatively shallow waters overlying most of the oyster reefs is influenced considerably by fresh water dilution during periods of heavy rainfall. Despite the low amplitude of tides, the salinity of the water at the mouths of the streams showed variation, associated with tide, during periods of low run-off.

To illustrate variations in the extent of fresh water dilution, the percent occurrence of various salinities (% of samples in particular salinity range) is presented in Table 2.

In area A, salinities from all stations diminished during periods of heavy rainfall. The greatest reduction occurred at station 1 near the mouth of a stream. In area A, therefore, salinities from station 1 and from station 5 which was remote from fresh water influence, were compared. In areas B and M, salinity at all stations was influenced to about the same extent by run-off. In these two areas salinities obtained from all stations during periods of low and high fresh water influence were compared.

In area A, approximately 52 percent of the salinities from the station near the stream were less than 10.1 ppt and 31.5 percent were less than 5.1 ppt. Only 5 percent of the salinities at the station remote from fresh water influence were less than 10.1 ppt and 58 percent were greater than 20 ppt. In area B, during the period of fresh water influence all of the salinities were less than 10.1 ppt. During the period of low fresh water influence, 90 percent of the salinities were greater than 10.1 ppt and 32 percent were greater than 20 ppt. Throughout the period of study, salinities in area M were lower than normal. During the period of highest fresh water influence, all of the salinities were less than 5.1 ppt and 75 percent were

TABLE 2. SALINITY OF THREE AREAS AT HIGH AND LOW FRESH WATER INFLUENCE

Salinity range, ppt	Percent of Samples in Range					
	Area A[a]		Area B[b]		Area M[b]	
	High	Low	High	Low	High	Low
<1.8	10.5	0	0	0	75	0
1.8- 5.0	21	0	41	0	25	8
5.1-10	21	5	59	10	0	92
10.1-20	26	37	0	58	0	0
20.1-32	21	58	0	32	0	0

[a] Two stations.
[b] Two periods.

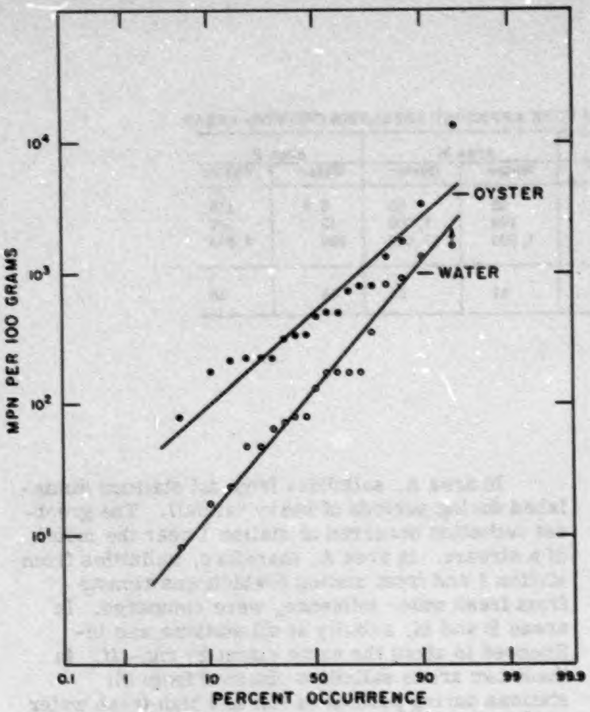

Figure 11. Influence of low salinity on water and oyster coliform MPN's at station 1, area A (see Table 2)

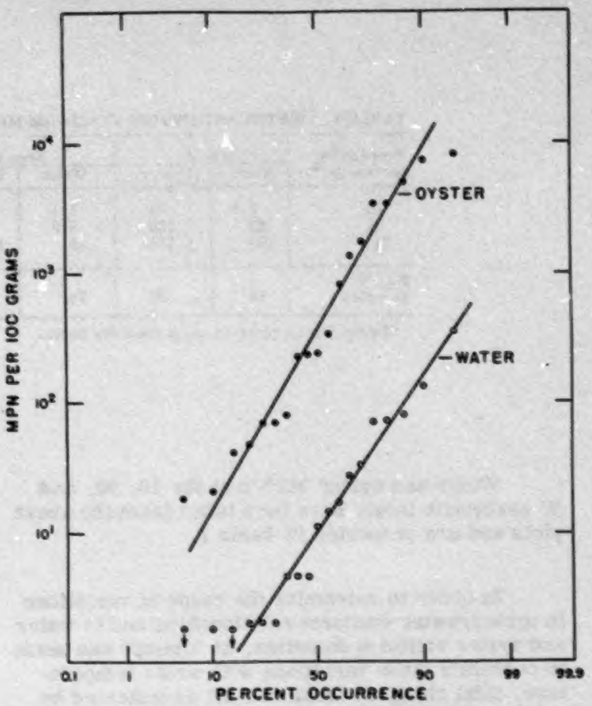

Figure 12. Influence of high salinity on water and oyster coliform MPN's at station 5, area A (see Table 2)

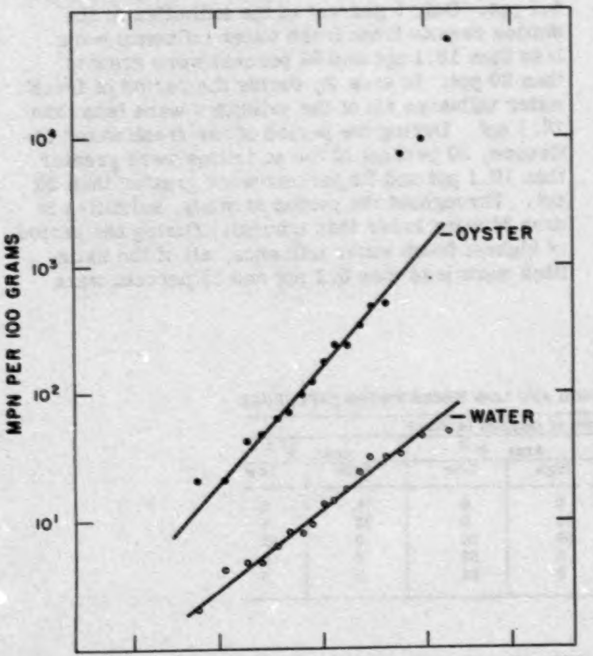

Figure 13. Influence of low salinity on water and oyster coliform MPN's in area B (see Table 2)

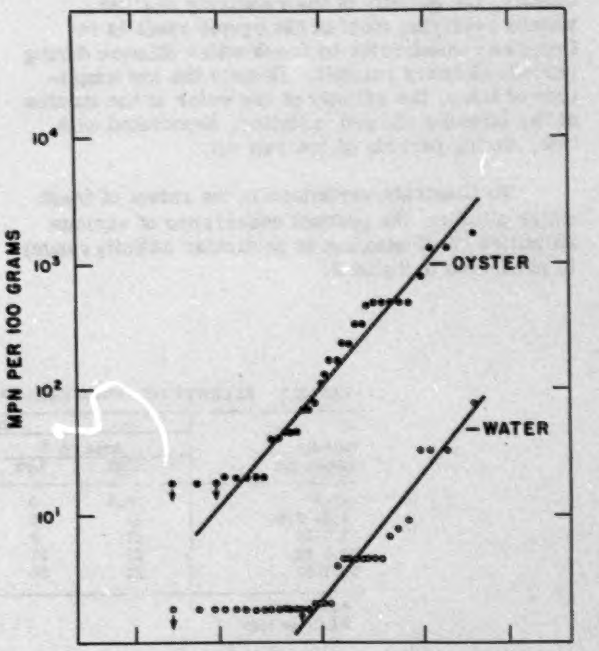

Figure 14. Influence of high salinity on water and oyster coliform MPN's in area B (see Table 2)

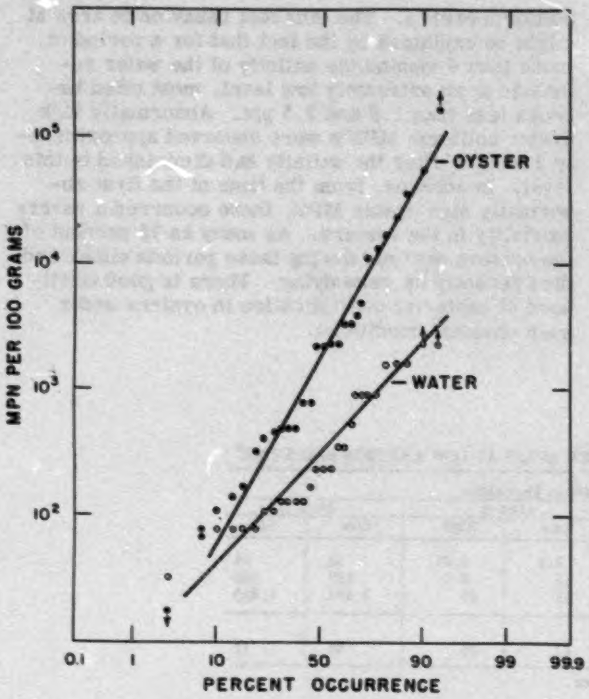

Figure 15. Influence of low salinity on water and oyster coliform MPN's in area M (see Table 2)

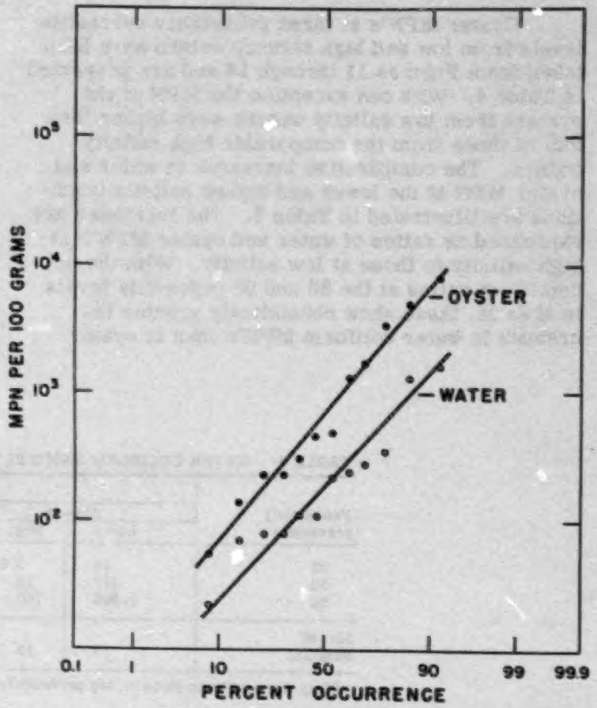

Figure 16. Influence of high salinity on water and oyster coliform MPN's in area M (see Table 2)

less than 1.8 ppt. During the period of lowest fresh water influence none of the salinities were less than 1.8 ppt and 92 percent were within the range 5.1 to 10 ppt. In area P, salinities were at different levels during the two periods, but an insufficient number of samples in each salinity category prevented a conclusive analysis of results.

In order to illustrate the influence of run-off on water and oyster coliform content and on oyster/water coliform relationships, water and oyster MPN's obtained in areas A, B, and M under the salinity conditions shown in Table 2, were plotted on probability paper. These plots are presented in Figures 11 through 16. In all areas, run-off, as indicated by salinity changes, influenced water and oyster coliform content and oyster/water coliform relationships.

Water coliform MPN's obtained under conditions of low and high salinity have been taken from Figures 11 through 16 and are presented at three probability percentile levels in Table 3. In all three areas, the low salinity water showed the higher MPN's. The greatest difference between low and high salinity waters occurred in area A, where the geometric mean MPN's were 130 and 10 respectively. In area B the mean water coliform MPN increased from 2.2 at high salinity to 12 at low salinity. In area M the increase was from 180 to 320.

The influence of run-off on oyster/water coliform relationships is demonstrated by a comparison of Figures 11 through 16. In all instances water and oyster lines under conditions of high salinity show the greatest degree of parallelism and the widest spread between the water and oyster results. The higher water coliform content under conditions of low salinity in the area was reflected in a higher coliform content of the oyster; however, increases in the water and oyster MPN's were disproportionate, i.e., the increases in the water MPN's were greater.

Oyster MPN's at three probability percentile levels from low and high salinity waters have been taken from Figures 11 through 16 and are presented in Table 4. With one exception the MPN of the oysters from low salinity waters were higher than that of those from the comparable high salinity waters. The comparative increases in water and oyster MPN at the lower and higher salinity conditions are illustrated in Table 5. The increases are expressed as ratios of water and oyster MPN's at high salinity to those at low salinity. With the exception of ratios at the 50 and 90 percentile levels in area M, these show consistently greater increases in water coliform MPN's than in oyster coliform MPN's. The different behavior in area M might be explained by the fact that for a period of more than 4 months the salinity of the water remained at an extremely low level, most often between less than 1.8 and 2.5 ppt. Abnormally high oyster coliform MPN's were observed approximately 2 months after the salinity had diminished to this level. In addition, from the time of the first abnormally high oyster MPN, there occurred a severe mortality in the oysters. As many as 75 percent of the oysters dredged during these periods either had died recently or were dying. There is good likelihood of bacterial multiplication in oysters under such physical conditions.

TABLE 3. WATER COLIFORM MPN'S IN THREE AREAS AT LOW AND HIGH SALINITIES[a]

Probability percentile	MPN in Percentile					
	Area A		Area B		Area M	
	Low	High	Low	High	Low	High
10	14	0.68	2.9	0.21	43	24
50	130	10	12	2.2	320	180
90	1,200	160	53	23	2,400	1,400
No. of Samples	19	19	17	34	28	12

[a]Data derived from plots on log probability paper.

TABLE 4. OYSTER COLIFORM MPN'S IN THREE AREAS AT LOW AND HIGH SALINITIES[a]

Probability percentile	MPN in Percentile					
	Area A		Area B		Area M	
	Low	High	Low	High	Low	High
10	100	15	19	12	61	70
50	500	370	160	110	1,900	680
90	2,600	9,800	1,400	1,000	55,000	6,600
No. of Samples	19	19	17	34	28	12

[a]Data derived from plots on log probability paper.

TABLE 5. RATIO LOW/HIGH SALINITY WATER AND OYSTER MPN'S

Probability percentile	MPN in Percentile					
	Area A		Area B		Area M	
	Water	Oysters	Water	Oysters	Water	Oysters
10	21	6	13.3	1.6	1.8	0.9
50	13	1.4	5.4	1.5	1.8	2.8
90	7.5	0.3	2	1.4	1.7	8.3

These results indicate that oyster feeding, as indicated by relative oyster/water coliform content, was more consistent and at a higher rate under conditions of high salinity. Under conditions of low salinity the feeding rate was not only lower but also erratic, as indicated by the degree of convergence of water and oyster lines at the high MPN station 1 in area A(Figure 11) and at the low values in area M (Figure 15).

In area A and B bottom deposits were examined to determine their coliform content. The method of collection and examination is described in the section on materials and method. Table 6 presents the coliform MPN's of water and bottom deposits at three probability percentile levels.

The coliform MPN's of bottom deposits were consistently higher than those of the corresponding water. At the 50 percentile level the bottom deposit MPN was 37 times the water MPN in area A and 60 times the water MPN in B. These results indicate that, on resuspension during periods of turbulence, bottom deposits could increase the coliform content of water. The coliform content of oysters actively feeding during these periods also would be increased.

Compliance With Recognized Coliform Standards

The Public Health Service Manual of Recommended Practice for the Sanitary Control of the Shellfish Industry[5] contains bacteriological criteria for shellfish and shellfish growing waters. The manual stipulates that the median water coliform MPN in an approved area shall not exceed 70 per 100 ml, and ordinarily not more than 10 percent of the samples should exceed MPN 230 in those portions of the area most probably exposed to contamination. These bacteriological findings must be supported by a sanitary survey which indicates that pathogenic micro-organisms do not reach the area in dangerous concentrations. Furthermore, the foregoing limits need not be applied if it can be shown, by a detailed study, that the coliform organisms are not of direct fecal origin and do not indicate a public health hazard. The above standards apply to all shellfish growing areas throughout the United States. In regard to the eastern oyster the current edition of the manual recognizes two standards, one applicable to oysters as harvested from approved areas in the North and Middle Atlantic regions and the other to oysters as harvested from the Gulf States. As set forth in the Manual, oysters as harvested from approved areas in the North and Middle Atlantic regions ordinarily should not exceed a coliform MPN of 230 per 100 grams of meat, although a few samples may approach or exceed 2400 per 100 grams. If the latter value is exceeded in two consecutive samples, the State shellfish control agency should investigate to determine the probable cause. Oysters as harvested from approved areas of the Gulf States are not expected to routinely meet the standard of 230 per 100 grams, even though they are harvested from waters of sanitary quality equivalent to that of waters from the North and Middle Atlantic regions. Oysters from the Gulf area normally are expected to have a coliform MPN of less than 2400 per 100 grams. If this value is exceeded in two consecutive samples, the State control agency should investigate to determine the probable cause. In the previous edition of the Manual [6] the limiting MPN of 230 standard was applicable in all areas and applied to oyster shell stock sampled at the growing areas and to shell stock and shucked oysters at the point of shucking. This standard was based primarily on studies conducted in the North and Middle Atlantic regions.

TABLE 6. WATER AND BOTTOM DEPOSIT COLIFORM MPN'S IN TWO APPROVED SHELLFISH GROWING AREAS

Probability percentile	MPN in Percentile			
	Area A		Area B	
	Water[a]	Bottom[b] deposits	Water	Bottom deposits
10	2.1	140	1.1	92
50	25	940	5.9	360
90	300	6,400	33	1,400
No. of samples	84	26	76	52

[a] MPN per 100 ml.

[b] MPN per 100 ml settleable solids.

TABLE 7. COMPLIANCE OF GULF COAST WATERS AND OYSTERS WITH RECOMMENDED BACTERIOLOGICAL LIMITS

Coliform group, MPN	Percent of samples in MPN group			
	Area A	Area B	Area M	Area P
	Water			
70 or less	70	96.5	28	63
More than 230	13	0.3	45	12.5
	Oysters			
Less than 230	36	60	23	24
230 to 2400 (inclusive)	51	31.5	42	64
Greater than 2400	13	8.5	35	12

The degree of compliance of oyster and water results obtained on the Gulf Coast with the currently recommended bacteriological criteria is presented in Table 7.

Compliance of Water Samples

Area B showed a high degree of compliance, approximately 97 percent of the MPN's were less than 70. Areas A and P conformed with the median MPN of 70 but slightly exceeded the 10 percent limit recommended for MPN 230. Approximately 13 percent of the samples for both areas showed MPN's in excess of this value. Waters from area M did not conform with the recommended criteria, since only 28 percent of the MPN's were 70 or less and 45 percent exceeded MPN 230. It should be emphasized that the study areas are approved for the growing and direct marketing of oysters by the state regulatory authorities. The above results are presented to illustrate variations, in the degree of compliance, that might be expected in waters from the four areas and are not intended to imply that the areas should not be approved.

Compliance of Oyster Samples

Results of the present study are in general agreement with previously presented data[7, 8] which show that oysters as harvested from approved areas on the Gulf Coast cannot be expected to routinely meet the limiting coliform MPN of 230 applicable to the North and Middle Atlantic regions. In the four areas studied a minimum of 40 percent (area B) and a maximum of 77 percent (area M) of the samples yielded MPN's equal to or greater than 230. On the basis of data presented at the 1956 Shellfish Sanitation Workshop,[9] it has been concluded that oysters as harvested from approved Gulf areas might ordinarily have a coliform MPN of less than 2400 per 100 grams of meat. These data, representing samples collected only from area B during one spring season and one summer season, show that 90 percent of the samples yielded coliform MPN's of less than 2400. Results of the present study, based on samples collected from area A, B, and P throughout one or more oyster harvesting seasons, are in close agreement with the data presented at the Workshop. In area B approximately 91 percent of the MPN's were 2400 or less, whereas in area A 87 percent and in area P 88 percent of the MPN's were equal to or less than this value. Results obtained from area M were significantly different than those from the other three areas. In this area 35 percent of the samples collected throughout one harvesting season yielded MPN's in excess of 2400. This low degree of compliance with current Gulf Coast oyster standards correlates quite well with the low degree of compliance with recognized water standards.

DISCUSSION

A knowledge of the coliform levels that normally occur in shellfish growing waters remote from pollution and of the oyster/water relationships in such areas is necessary for the establishment of safe, reasonable standards for oysters at the point of harvesting. This report presents such data, gathered in four commercially important shellfish growing areas on the Gulf Coast.

Variations in oyster/water coliform relationships and in the coliform densities of oysters and waters were found. Probably the most important factor associated with these variations was run-off, which was reflected by fluctuations in salinity. Coliform densities of the waters were at a maximum under low salinity conditions.

As a result of the increased coliform content of the water during periods of run-off, oysters at this time usually showed higher coliform MPN's than during periods of high salinity. Increases in coliform content of the oysters were not proportionate, however, to water coliform increases due to inhibition of feeding activity under low salinity conditions. Our results confirm the findings of Loosanoff 10 to the extent that they show that under conditions of low salinity feeding by the eastern oyster is decreased and erratic.

Based on bacteriological results, three of the four areas complied with water coliform criteria recommended for approved growing areas. Waters from one of the areas, with a geometric mean MPN of 5.9 and with 96.5 percent of the MPN's 70 or less, showed a very high degree of compliance. Two others complied with the median MPN of 70, but slightly exceeded the 10 percent limit recommended for MPN 230. The fourth area, with a mean water MPN of 190 and with only 28 percent of the MPN's 70 or less, did not comply with recommended water bacteriological criteria. Throughout the period of study this area was subjected to abnormally high run-off. Since all of the areas are approved for the growing and direct marketing of oysters however, it must be assumed that the waters are free from dangerous concentrations of pathogenic organisms and that no health hazard exists.

Regardless of the degree to which waters complied with recommended criteria, oysters from all areas did not comply with limits recommended in the manual for eastern oysters at the point of harvesting or shucking in North or Middle Atlantic areas. Since the present study was conducted in four widely separated areas throughout one or more harvesting seasons and encompassed a wide variety of environmental conditions, it is reasonable to assume that the results are typical of the Gulf Coast Region. Results of the study indicate that compliance with the 230 coliform MPN limit recommended in the manual for eastern oysters is not possible in the Gulf Coast Area.

Since approximately 90 percent of the oyster samples from three of the four study areas yielded MPN's of 2400 or less per 100 grams of meat, the results of the present study indicate that the currently recommended coliform standard of 2400 applicable to Gulf Coast oysters as harvested is more reasonable than the standard of 230 formerly applied to the region. Although the present study indicates that the current standard is a more reasonable one, it has not been established that the 2400 MPN limit is an absolute criterion from the standpoint of attainability. This is emphasized by the fact that 35 percent of the oyster samples from one of the areas yielded MPN's in excess of 2400. Furthermore, there is a good possibility that two or more consecutive samples from any of the areas might exceed 2400, particularly during periods of increased activity or under turbulent conditions when bottom deposits, with their relatively high coliform content, are resuspended.

The current standard was established under the assumption that oysters harvested from approved Gulf Coast areas with a coliform MPN of 2400 or less per 100 grams of meat will be free from dangerous concentrations of pathogenic organisms. Since the likelihood of pathogenic organisms reaching an approved area is low, however, it is quite possible that oysters with a coliform index in excess of 2400 will also be safe for consumption. This possibility is recognized in the current recommended standards, i.e., nonconsecutive samples are permitted to exceed the 2400 limit. Although it is concluded that the present standard is more reasonable for the Gulf Coast, toleration of a 2400 coliform MPN level for Gulf Coast oysters in face of the fact that an MPN level of 230 is applied to oysters from northern waters would place some doubt on the validity of the entire coliform group as an index of pollution. As indicated in part II of this report, perhaps Escherichia coli, generally considered to be more indicative of pollution than the other coliform types, should be considered for use as an index organism.

PART IIA. CHANGES DURING HARVESTING, PROCESSING, AND STORAGE OF OYSTERS

SUMMARY

A field study has been conducted in four areas on the Gulf Coast throughout one or more oyster harvesting seasons to determine the bacterial changes that occur in oysters during harvesting, processing and storage.

The study involved the determination of the coliform and plate count content of oysters at various stages of harvesting and processing and after varying intervals of storage under simulated market conditions. The coliform and plate count content of mud and other materials washed from shell oyster samples at the point of harvesting on delivery to the shucking houses and just prior to commercial shucking were also determined. In addition, a number of stored lots were also examined to determine changes in their E. coli type I content.

In three of the areas where commercial lots of oysters could be traced directly from the reefs, substantial increases occurred in the coliform and plate count content of the oysters and the material adhering to their shells in the interval between harvesting and commercial shucking. Results indicate that these increases were primarily the result of bacterial multiplication in the body and body fluids during temporary storage of the shell oysters at temperatures ranging from 10° to 30° C for periods of 12 to 16 hours.

In all areas further coliform and plate count increases occurred during commercial shucking. In the areas studied, the shell oysters were not washed on arrival at the shucking houses and in most instances considerable quantities of mud and other bottom materials adhered to the shell stock. Substantial amounts of these materials were introduced into the shucked product by the methods of shucking. Although washing of the product prior to packing usually accomplished reductions in the coliform MPN's and plate counts, it was only in two areas that the bacterial content of the shucked product was reduced to a level approximating that of the shell stock prior to shucking. These results indicate that a thorough washing of the shell stock prior to shucking might eliminate much of the bacterial increase due to shucking and ultimately produce a packed product with lower bacterial content.

Data from the present study demonstrated that a detention time in the shuckers pots of approximately 2 hours did not result in increases in the coliform or plate count content of shucked oysters.

The study showed that the increases in coliform content and plate count of the packed product were primarily results of multiplication of the bacterial flora present within or on the shells of the oysters. Since the oysters were harvested from approved areas where the numbers of enteric pathogens if present would be extremely low and since the bacterial increases occurred at ambient air temperatures inhibitory to the growth of such pathogens, it is concluded that the sanitary significance of the increases would be minimal. Thus, the need for a more meaningful index organism is indicated.

Studies of the bacterial changes that occurred in shucked oysters stored at 2° to 12°C revealed two definite patterns of coliform and plate count changes and much better keeping qualities than those from the other three areas; however, the coliform and plate count content of oysters from all areas increased significantly during the first 4 days of storage.

In contrast to the substantial increases in the coliform and plate count content resulting from shucking or multiplication during storage, no significant changes in E. coli type I density occurred during storage. This would indicate the possible superiority of E. coli as an index of the sanitary quality of market oysters.

INTRODUCTION

The bacteriological examination of shellfish is important in evaluations of the sanitary quality of the commercial product as grown and of the changes

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in quality during harvesting, processing, and marketing. In assessments of the sanitary quality of shellfish, use has been made of two bacterial indicators. The coliform group of bacteria has been made of two bacterial indicators. The coliform group of bacteria has been used to indicate the presence of fecal pollution, both at the point of harvesting or shucking and at the market level, whereas the standard plate count has served as an index of unsatisfactory handling during processing, improper refrigeration, or mishandling of the product while in transit.

In conjunction with a bacteriological study of Gulf Coast oyster growing areas, the Shellfish Sanitation Laboratory conducted a study of the influence of commercial harvesting and processing on the coliform content and plate count of commercial lots of oysters, from the growing areas through the processing plants. Initially, the purpose of the study was to assess the significance of the currently accepted bacterial indices in evaluations of the sanitary quality of oysters as harvested from approved growing areas and as processed by accepted commercial practices. Early in the study it became apparent that use of the current indicator organisms, especially the coliform group, might be of doubtful value for assessment of the sanitary quality of Gulf Coast oysters at the market level. Therefore, the study was extended to include an investigation of the changes in coliform content and plate count of stored oysters with a view to assessing the significance of the indicator groups in evaluating the sanitary quality of oysters held under simulated market conditions.

As an integral part of the above program, the Laboratory participated in a cooperative study to evaluate the relative merits of the coliform group and *Escherichia coli* as indicators of the sanitary quality of market oysters. This study developed as a result of discussions held at the 1956 Shellfish Sanitation Workshop.[9] Because of the lack of a standard method of enumeration, the Workshop recommended that the Shellfish Sanitation Laboratory outline and submit for approval, by the cooperating agencies, a procedure for enumerating *E. coli*. Choice of the method submitted was based on an evaluation of four methods used in the enumeration of *E. coli*. The methods and results of this study are included in part IIB of this report.

MATERIALS AND METHODS

Collection of Samples

Samples of the eastern oyster, *Crassostrea virginica*, were collected from reefs and oyster houses in four areas on the Gulf Coast. The areas have been designated by the letters A, B, M, and P and are identical with the areas investigated during the course of the bacteriological study of shellfish growing areas described in part one of this report. Traced commercial lots of oysters were sampled, when possible, at the following stages of harvesting, processing, and storage.

1. As harvested from the reefs.

2. As landed at the oyster houses, 4 to 6 hours after harvesting.

3. Immediately before commercial shucking, 12 to 16 hours after harvesting.

4. As commercially shucked, a composite sample shucked into a sterile container by several shuckers.

5. On delivery to the packing room from shuckers' pots.

6. As packed.

7. During storage at refrigeration temperature. (Samples for storage consisted of 1 gallon lots of commercially packed oysters. The shellfish were stored at 2° to 12°C under dry refrigeration. It was recognized that the selected temperature was not optimum but might often be the temperature encountered during the commercial handling of oysters.)

Oyster samples were collected and transported in accordance with methods outlined in the American Public Health Association Recommended Procedures for the Bacteriological Examination of Shellfish and Shellfish Waters.[3]

To investigate the influence on the bacterial content of oysters by bottom muds and other materials adhering to the shells, examinations were made of washings of the shells at the point of harvesting, on landing, and just prior to commercial shucking. The method involved placing ten oysters in a sterile water-tight can, adding 1 liter of sterile, phosphate-buffered, dilution water, and then agitating the can at least 50 times with a continuous orbital motion. Care was taken to avoid violent motion that might break the outer edges of the shells and thus allow incorporation of shell liquor with the washings. Immediately after agitation at least 100 ml of washings were transferred to a sterile bottle and placed on ice until examined.

Bacteriological Methods

All samples of oysters and washings were examined for coliform and bacterial content by methods described in Recommended Procedures for the Examination of Shellfish and Shellfish Waters,[3] with the following modifications.

1. The dilution water was Butterfield's phosphate-buffered dilution water recommended in Standard Methods for the Examination of Water, Sewage and Industrial Wastes, Tenth edition.[11]
2. Dilution of the oysters was by weight rather than volume as recommended in the 1946 Shellfish Procedures.
3. Diluted oyster samples were ground in a Waring Blender for 60 seconds.

Coliform densities were determined by the multiple tube method (five tubes per dilution). Appropriate volumes of sample were inoculated into lauryl sulfate lactose (LSL) broth in a series of four decimal dilutions. The tubes were incubated for 24 and 48 hours at 35° ± 0.5°C. The presence of coliform organisms was confirmed by transferring cultures from all gas positive tubes to brilliant green lactose bile broth. Production of any amount of gas in the brilliant green lactose bile broth tubes within 24 or 48 hours of incubation at 35° ± 0.5°C was considered evidence of the presence of coliform organisms.

Results were expressed as the Most Probable Number (MPN) of coliforms per 100 grams of oyster meat and per 100 ml of washings.

Plate counts were determined by preparing duplicate tryptone glucose extract agar plates of appropriate volumes of sample. Plates were incubated for 48 hours at 35° ± 0.5°C and counted in accordance with methods described in Standard Methods for the Examination of Dairy Products, Tenth edition.[12] Results were expressed as plate count per gram of oyster meat or per ml of washings.

In addition to examination by the Standard Methods for coliform content and plate count, a number of oyster samples were submitted to E C tests to determine their E. coli content. Since there is no standard method for the enumeration of this organism, four of the more promising methods were evaluated. Based on the results of this evaluation, the E C liquid confirmatory test was the method of choice. A complete description of the methods and the results of the evaluation study are presented in part IIB of this report.

RESULTS

During the study, 44 sampling tours were made in area A, 32 in area B, 17 in area M, and 6 in area P. In areas A and B the sampling period extended from June 1956 through February 1958 and in area B from March 1956 through January 1958. In areas M and P, samples were collected over a period extending from November 1957 through April 1958.

Changes in Coliform MPN's and Plate Counts during Harvesting and Processing

A complete tabulation of all data will be found in the appendix. In area M a scarcity of oysters made it necessary that harvesters visit several reefs and remain on the harvesting grounds for intervals of 1 to 5 days in order to obtain economically important quantities of oysters. Consequently, it was not possible to establish a satisfactory schedule for obtaining samples at the reef or on delivery to the shucking houses. In this area the first sample available was shell oysters just prior to commercial shucking. In area P, where oysters were collected from reefs close to the processing plants, the time interval between harvesting and delivery was not considered significant. Hence, samples were not collected "as landed".

Probability plots of coliform MPN's and plate counts obtained from shell and shucked oyster samples and from samples of shell oyster washings collected at the various stages of harvesting and processing were prepared in accordance with methods described by Velz.[4] Oyster coliform MPN's taken from probability plots, at three percentile levels, are presented in Table 8.

In areas A, B, and P, significant increases in coliform content occurred during the interval between harvesting and commercial shucking. In this interim, the geometric mean (50 percentile level) of the shell oysters increased approximately 2, 100, and 10 fold in areas A, B, and P, respectively. In all areas the commercial shucking process resulted in further substantial increases in the coliform content of oyster. A comparison of the mean MPN's of oysters before and after shucking reveals that the commercial shucking process effected increases in the mean MPN's of approximately 2, 3, 2, and 7 fold in areas A, B, M, and P, respectively.

TABLE 8. COLIFORM MPN'S AT 10, 50, and 90 PROBABILITY PERCENTILE LEVELS

Probability percentile	Shell oysters			Shucked oysters		
	Reef	As landed	Before shucking	As shucked	Shuckers pots	As packed
Area A						
10	54	120	96	170	190	220
50	380	620	900	1,400	1,500	1,500
90	2,700	3,200	8,400	11,000	13,000	11,000
Area B						
10	10	62	1,200	5,400	2,200	1,500
50	130	800	12,000	33,000	38,000	11,000
90	1,800	10,000	120,000	200,000	350,000	74,000
Area M						
10			2,300	13,000	12,000	8,000
50			38,000	90,000	68,000	60,000
90			580,000	640,000	390,000	460,000
Area P						
10	110		1,800	6,200	1,700	2,300
50	540		5,500	35,000	14,000	8,000
90	2,600		17,000	210,000	110,000	28,000

In commercial practice on the Gulf Coast the average detention time of the shucked product in the shuckers' pots is approximately 2 hours. To determine the influence of this detention on the bacterial content of oysters, samples were collected from shuckers' pots on delivery to the packing room. Although results from the four areas were variable, a comparison of MPN's from the "as shucked" and "shucker's pot" samples shows that detention in the shuckers' pots did not result in substantial increases in the coliform content of oysters in any of the areas.

In all areas except A, the mean coliform MPN's of the "as packed" product were significantly lower than the mean MPN's of the "as shucked" samples, demonstrating that in areas B, M, and P washing of the shucked product prior to packing was effective in removing considerable quantities of the coliform organisms introduced by the shucking process. In area A, the mean coliform MPN of the "as packed" samples was approximately twice that of samples collected prior to shucking and comparable to the mean MPN of the "as shucked" sample.

Plate counts of oysters at three probability percentile levels are presented in Table 9. In area B, plate count values from reef oysters could not be stated with confidence because of the abnormal distribution of results, which could not be correlated with season or with measured fluctuations in water temperature or salinity. A probability plot of these counts is presented in Figure 17. As indicated by the plot, there was a strong indication of two distinct bacterial density groups. Approximately two-thirds of the plate counts fell into a group showing relatively little variation and good conformity to a straight line.

The upper one-third of the counts, although higher and more variable, were also arrayed along a straight line of probability. Reef and as landed samples in area M and as landed samples in area P were not collected for reasons previously stated.

In areas A and P the mean plate count increased approximately 2 and 3 fold, respectively, during the period between harvesting and shucking. In area B the mean count increased about 5 fold in the interim between landing and shucking. Increases

TABLE 9. PLATE COUNTS AT 10, 50, AND 90 PROBABILITY PERCENTILE LEVELS

Probability percentile	Shell oysters			Shucked oysters		
	Reef	As landed	Before shucking	As shucked	Shuckers pots	As packed
Area A						
10	570	500	570	1,300	880	2,000
50	1,200	1,100	1,900	5,800	4,100	6,600
90	3,600	2,500	6,400	25,000	19,000	36,000
Area B						
10		1,500	2,000	8,400	7,000	3,700
50		6,000	30,000	80,000	76,000	25,000
90		25,000	450,000	860,000	860,000	170,000
Area M						
10			3,000	9,000	10,000	6,500
50			44,000	140,000	88,000	80,000
90			740,000	9,000,000	740,000	990,000
Area P						
10	390		1,500	6,100	2,000	3,200
50	1,600		5,000	18,000	19,000	12,000
90	7,000		17,000	52,000	180,000	56,000

in the geometric mean of plate counts resulting from commercial shucking were comparable in all areas, i.e., approximately 3 fold in areas A, B, and M and about 4 fold in area P. These results show a close correlation with the mean coliform increases resulting from commercial shucking.

As in the case of coliform results, detention in the shuckers' pots did not cause a substantial increase in the plate counts. In most instances the plate counts of samples from shuckers' pots were lower than counts from the "as shucked" samples, probably reflecting the exclusion of shell liquor from the former samples. Changes in the plate counts resulting from washing of the oyster meats prior to packing showed considerable area to area variation, which correlated well with the coliform results.

Geometric mean coliform MPN's and Plate Counts of shell oyster washings are presented in Table 10.

Substantial increases in the mean coliform content of the washings occurred in the interval between harvesting and shucking. Increases in coliform content ranged from 10 fold in area A to approximately 230 fold in area B.

Although of a lower magnitude than coliform increases, the plate counts of the washings also showed significant changes in the interval between harvesting and shucking. The counts increased approximately 6, 8, and 25 fold in the three areas tested. The geometric mean count from reef samples in area B could not be stated with confidence because of an abnormal distribution of results. The probability plot of the results is presented in Figure 18. The distribution of these results is similar to the distribution of plate counts from reef oyster samples (see Figure 17).

Bacterial Changes in Oysters During Storage

Samples from a total of 23 one-gallon lots of commercially shucked and packed oysters were examined for coliform content and plate count. Samples from the 1-gallon lots were collected at the time of commercial packing and after varying intervals of laboratory storage under conditions of dry

TABLE 10. GEOMETRIC MEAN COLIFORM MPN'S AND PLATE COUNTS OF SHELL OYSTER WASHINGS

	Coliform MPN/100 ml[a]			Plate count/gram[a]		
Area	Reef	As landed	Before shucking	Reef	As landed	Before shucking
A	220	1,500	2,200	3,900	5,300	22,000
B	180	4,200	41,000		30,000	260,000
M			86,000			180,000
P	320		19,000	1,800		45,000

[a]Data derived from log probability plots.

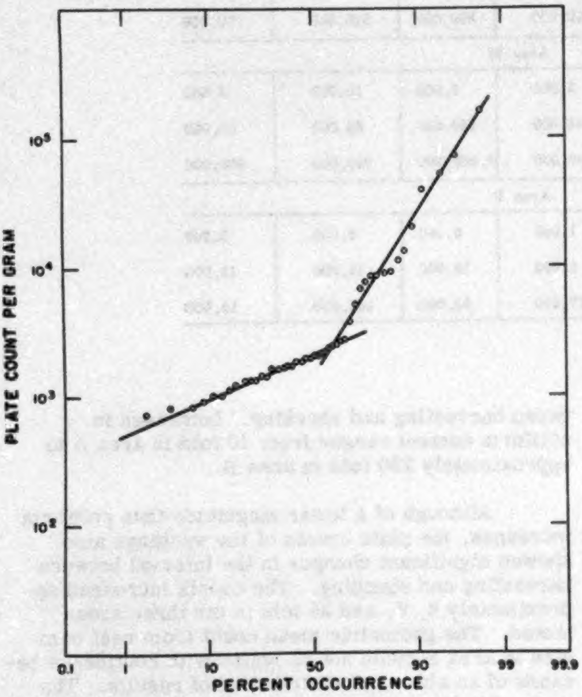

Figure 17. Plate counts for reef oysters from area B

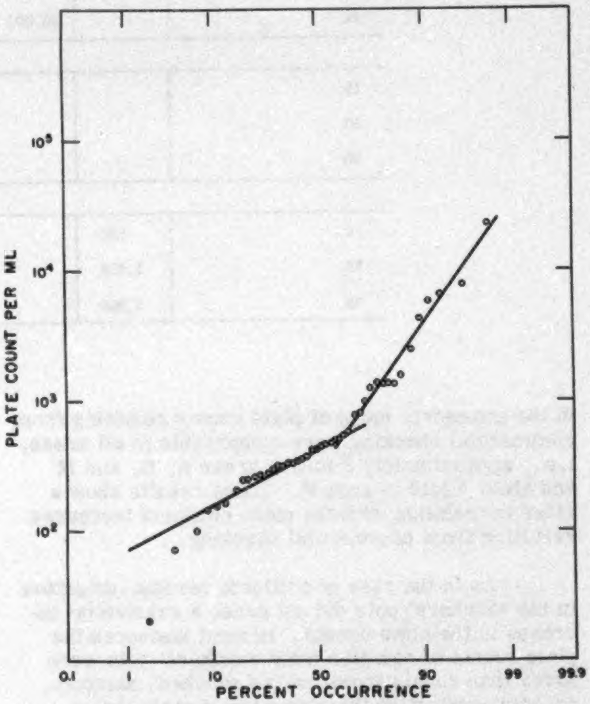

Figure 18. Plate counts for washings of reef oysters from area B

refrigeration described in the section on materials and methods. Although the time and material involved prevented the determination of the changes in the E. coli type I content on all lots, such changes were determined on 13 of the 1-gallon lots.

Table 11 presents the geometric mean coliform MPN's and plate counts obtained. Since pH changes have been considered as indicative of the degree of spoilage, average pH values are included in the table.

The comparative patterns of coliform and plate count changes in oysters from area A and from areas B, M, and P are shown in Figures 19 and 20. Results from areas B, M, and P were similar and therefore were combined. Results from area A were significantly different and therefore are presented separately.

TABLE 11. GEOMETRIC MEAN COLIFORM MPN'S AND PLATE COUNTS OF SHUCKED OYSTERS STORED AT 2° to 12°C

Storage, days	Number of samples	Average pH	Coliform MPN per 100 grams	Plate count per gram
AREAS B, M and P				
0	14	6.6	16,000	37,000
1-2	17	6.2	48,000	72,000
3-4	11	6.1	52,000	120,000
5	7	6.0	120,000	180,000
6-7	12	6.0	440,000	530,000
8-9	10	5.8	710,000	600,000
11	6	5.5	4,800,000	4,200,000
Area A				
0	5	6.7	4,500	6,300
1-2	8	6.3	6,700	12,000
3-5	9	6.1	14,000	22,000
6-9	9	6.0	16,000	110,000
10-12	6	5.9	59,000	81,000
14-15	4	5.8	26,000	91,000
18-23	4	5.9	4,400	8,100

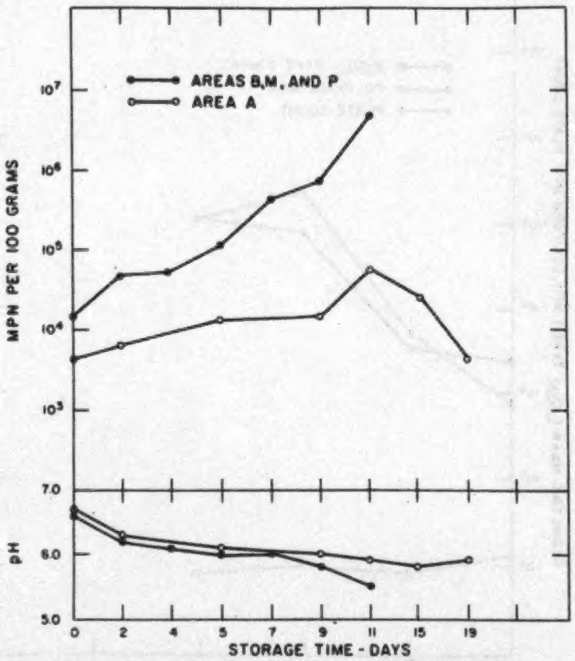

Figure 19. Effects of storage at 2° to 12° C on pH and coliform content of shucked oysters from four growing areas

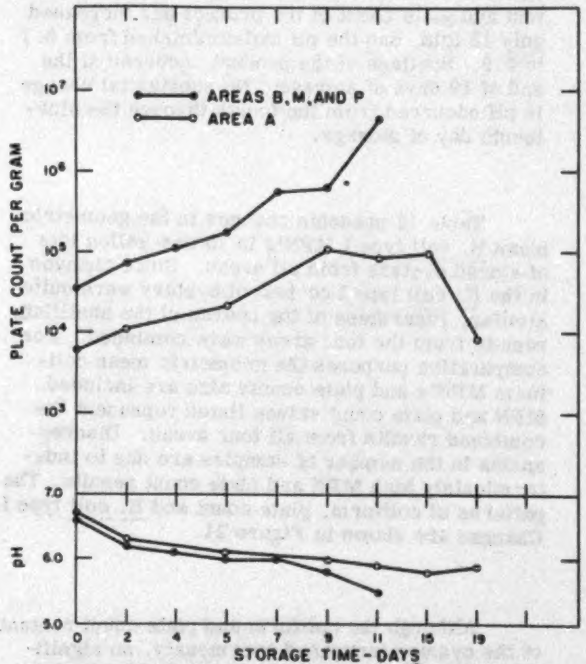

Figure 20. Effects of storage at 2° to 12° C on pH and plate count of shucked oysters from four growing areas

TABLE 12. GEOMETRIC MEAN E. COLI TYPE I MPN'S, COLIFORM MPN'S, AND PLATE COUNTS FROM SHUCKED OYSTERS STORED AT 2° to 12°C

Storage, days	E. coli type I		Coliforms		Plate count	
	Number of samples	MPN/100 grams	Number of samples	MPN/100 grams	Number of samples	Count per gram
0	12	140	9	7,200	11	24,000
2	4	100	4	50,000	4	34,000
5	4	110	3	2,200,000	4	720,000
11	10	76	10	1,100,000	8	1,100,000

From the table and figures it is apparent that the rate of coliform and plate count increases was much higher in stored oysters from area B, M, and P. Oysters packed in these three areas showed progressive deterioration and enormous increases in coliform content and plate counts during 11 days of storage. After 11 days, the mean coliform MPN had increased approximately 300 fold, the pH had diminished from 6.6 to 5.5, and the oysters were no longer in saleable condition. Oysters packed in area A and stored under the same conditions deteriorated much more slowly. After 11 days of storage, oysters from the area were still in saleable condition, the coliform content and plate count of the product had increased only 13 fold, and the pH had diminished from 6.7 to 5.9. Spoilage of the product occurred at the end of 19 days of storage. No substantial change in pH occurred from the fourth through the nineteenth day of storage.

Table 12 presents changes in the geometric mean E. coli type I MPN's in 13 one-gallon lots of stored oysters from all areas. Since changes in the E. coli type I content of oysters were quite similar, regardless of the source of the shellfish, results from the four areas were combined. For comparative purposes the geometric mean coliform MPN's and plate counts also are included. MPN and plate count values listed represent the combined results from all four areas. Discrepancies in the number of samples are due to indeterminately high MPN and plate count results. The patterns of coliform, plate count and E. coli type I. Changes are shown in Figure 21.

Although the coliform and plate count content of the oysters increased enormously, no significant increases in the E. coli type I content of the product occurred as a result of 2, 5, or 11 days of storage at 2° to 12° C.

DISCUSSION

Results of the study of oyster harvesting and processing show that an important increase in the coliform and plate count content of oysters from areas A, B, and P occurred in the interim between harvesting and shucking. Indications are that substantial bacterial increases also occurred in area M during this interval. Although it was not possible to collect samples of oysters at the reefs or on delivery to the shucking houses in area

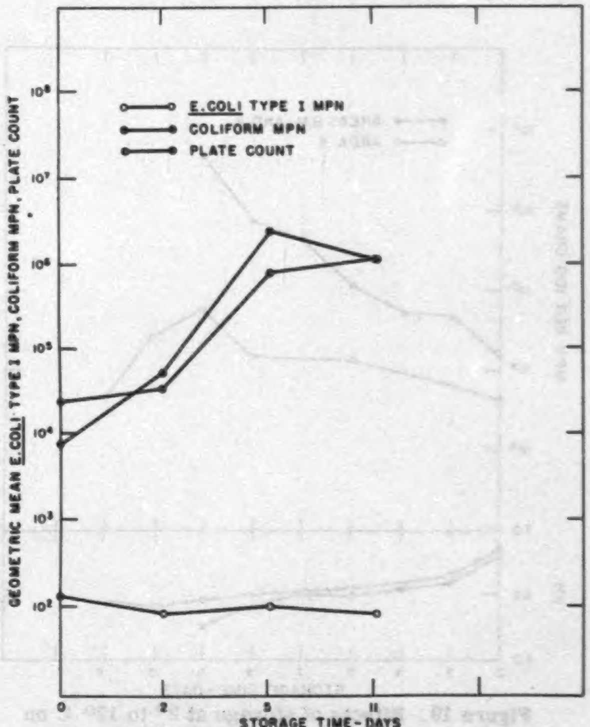

Figure 21. Effects of storage at 2° to 12° C on E. coli type 1, coliform, and plate count of shucked oysters

M, it can be reasonably assumed that the geometric mean MPN of 38,000 and mean plate count of 44,000 (Tables 8 and 9) obtained from samples collected just prior to commercial shucking represent a substantial increase during the 1 to 5 day interval that the oysters were held on the harvesting boats. In one section of this harvesting area we were able to examine 51 samples of oysters immediately after removal from commercial beds. The geometric mean coliform MPN of these samples was found to be 1000 per 100 grams of meat and the mean plate count 3000 bacteria per gram. The other section of the area was not investigated by us because of the distances involved; however, the State of Louisiana examined a total of 49 samples of oysters as harvested during the course of a sanitary survey conducted in 1948 and 1949.[13] The average MPN of their samples was found to be 110 coliforms per 100 grams of meat. By comparison, the average coliform MPN of oysters from this same area collected from Louisiana harvesting boats as they arrived at oyster houses after a maximum of 2 days on the harvesting grounds showed an average coliform MPN of 5400. Thus, we can assume that the mean MPN of 38,000 and mean plate count of 44,000 obtained from shell oysters samples collected just prior to commercial shucking in area M represents a continuous increase that correlates quite well with the time interval between harvesting and shucking.

Since there were no discernible sources of pollution and since all shell stock samples collected prior to commercial shucking were returned to the laboratory and thoroughly scrubbed and opened aseptically, it is concluded that increases in the coliform content and plate count of the oysters during the interval between harvesting and shucking were primarily the result of bacterial multiplication in the body fluids and bodies of the oysters. Further supporting the thesis that the bacterial increases were due to multiplication was the fact that air temperatures that prevailed throughout the period of study were conducive to bacterial multiplication during most of the oyster harvesting season. Even in the winter months, the outside air temperatures during the day exceeded 10°C most of the time. Day and night temperatures in the oyster houses ranged from 10° to 25°C throughout most of the oyster season. It is known from storage experiments that the coliform MPN's and plate counts of oysters increase at temperatures ranging from 2° to 12°C.

The coliform MPN's and plate counts of material adhering to the shells of oysters increased considerably during the interim between harvesting and shucking. These increases were also apparently the result of multiplication, since the shell oysters were not exposed to sources of contamination. These increases started on the harvesting boats and continued during overnight storage on shucking benches or storage bins.

Although the bacterial content of the material adhering to shell oysters had increased considerably in the interval between harvesting and shucking, there were no indications that this material contributed significantly to the bacterial content of the meats of oysters during storage as shell stock. Shell oysters washed in flowing water containing from 20 to 200 ppm of available chlorine and stored overnight on shucking benches showed no less multiplication in plate count and only slightly less increase in coliform content than comparable unwashed shell stock samples.

In all four areas the commercial shucking process resulted in further substantial increases in the coliform and plate count content of oysters. These increases can be attributed in part to contamination of the shucked product by material adhering to the outside of the shells. In commercial practice, in the areas studied shell oysters were not washed on arrival at the shucking houses and the methods of shucking introduced considerable quantities of the material adhering to the shells into the shucked product.

Although washing of the shucked oysters prior to packing usually reduced the coliform and plate count content, it was only in areas B and P that the coliform MPN's and plate counts of the packed product were reduced to levels approximating those obtained from shell stock samples collected just prior to commercial shucking. These results suggest that a thorough washing of the shellstock at the houses might eliminate much of the bacterial increase that occurs during shucking.

In all areas studied the average detention time in the shuckers pots was about 2 hours. Data collected in the present study show that no significant increases occurred in coliform content or plate count of oysters during this detention time.

On the basis of data presented, the most significant increases in the coliform MPN's and plate counts of the oysters packed for the market were directly attributable to bacterial multiplication in the bodies and body fluids of oysters in the interval between harvesting and shucking. These bacterial increases represent primarily multiplication of the bacterial flora in oysters grown in approved areas where the numbers of pathogenic organisms, if present, are very low. Consequently, there is little likelihood that the increased MPN's and plate counts are of sanitary significance. These results suggest, however, the need for a more meaningful indicator organism, such as that applied to market oysters from the Gulf Coast.

Results obtained from commercially packed oysters stored at dry refrigeration temperatures of 2° to 12°C revealed that the bacterial changes that occur in packed oysters from areas B, M, and P are quite different from those that occur in packed oysters from area A. From the time of packing, the coliform MPN's and plate counts of oysters from areas B, M, and P increased rapidly and consistently, with spoilage occurring after 11 days of storage. In oysters from area A the coliform and plate count content increased at a much lower rate during the 11 days of storage, and spoilage did not occur until after 19 days. Although the rates of increase were different, the coliform MPN's and plate counts of oysters from all areas had shown significant increases after 4 days of storage. The fact that the coliform increases occurred at temperatures inhibitory to the growth of enteric pathogens would tend to negate the significance of the entire coliform group as an index of the sanitary quality of stored oysters. In contrast, the number of *E. Coli* organisms, a type generally more often associated with fecal contamination than the other coliform types, remained relatively unchanged during processing and throughout storage.

The above results indicate the superiority of *E. coli* type I, as opposed to the entire coliform group, as an index of the sanitary quality of market oysters. The presented data do not, however, preclude the possibility that other members of the coliform group may have sanitary significance. Based on data presented at the 1958 Shellfish Sanitation Workshop,[14] it was concluded that the entire coliform group was not a meaningful indicator of pollution in shucked oysters at the market level. Consequently, the Workshop recommended the adoption of an interim standard based on the E C gas positive, "fecal coliform MPN", as an index of pollution and the plate count as an index of contamination during processing or of bacterial increases due to faulty handling or refrigeration during transit or marketing.

Comparative Behavior of Pathogenic and Indicator Organisms in Stored Shucked Oysters

In the storage study already described, it was established that the *E. coli* type I content of eastern oysters held at 2° to 12°C remained relatively unchanged throughout a period of 11 days whereas the coliform content and plate count increased progressively and significantly during this time. It was concluded that *E. coli* type I would be the index of choice in assessing the sanitary quality of eastern oysters at the market level. This conclusion was based on the assumption that enteric pathogens would behave in a parallel manner under similar storage conditions. In order to verify this assumption, a series of seven experiments was conducted to determine simultaneously the pattern of density change for three strains of salmonella, *E. coli* type I, the coliform group, and the plate count, in eastern oysters stored at 4° to 7°C and at 20°C.

MATERIALS AND METHODS

Seven gallons of shucked oysters were obtained from several commercial sources. Since these oysters usually had a relatively high coliform and *E. coli* content, it was only necessary to contaminate them with enteric pathogens. A suspension of equal concentrations of *S. derby*, *S. infantis*, and *S. newport* calculated to give the oysters an estimated MPN of 10,000 salmonella per 100 grams was added to 500 ml of sterile distilled water. This suspension was added to a gallon of oysters in a sterile enamelware container. After mixing and soaking period of 15 minutes, the oysters were drained in a colander and placed in containers for storage.

Condition of Storage

Refrigerated storage was in a household-type refrigerator adjusted to maintain a temperature of approximately 5°C. The temperature of the refrigerator ranged between 4° and 7°C. Appropriate amounts of oysters also were stored at 20°C, in an attempt to simulate adverse conditions that might occur in commercial handling as a result of improper refrigeration. This storage was accomplished in a specially equipped unit. which controlled the temperature within a variation of ± 1°C. Storage of the shellfish was continued until physical examination indicated that incipient spoilage had occurred.

Bacteriological Procedures

Procedures for the enumeration of *E. coli*, the coliform group, and the plate count have been described previously in this report. The method of salmonella determination was as follows. The sample was inoculated into lactose broth, and five tubes were used per dilution in a series of at least four decimal dilutions. After an incubation period of 24 hours at a temperature of 35° ±0.5°C, 0.1-

ml aliquots of material from each lactose tube was transferred into brilliant green tetrathionate broth. After 24 hours incubation at 35° ± 0.5°C, streakings from the broth were made on bismuth sulfite agar plates, which then were incubated at 35° ± 0.5°C for 24 to 48 hours. Colonies resembling salmonella were picked from the plates and identified on the basis of their reaction in Russell's double sugar and on the basis of results of agglutination tests with appropriate salmonella group antisera. The results of tests for salmonella, coliforms, and E. coli were expressed in terms of the MPN's per 100 grams of oyster meats. The standard plate count was expressed in terms of organisms per gram of meat.

TABLE 13. MEDIAN COLIFORM, SALMONELLA, AND E. COLI MPN'S AND STANDARD PLATE COUNT IN STORED SHUCKED OYSTERS

Storage, days	At 4°-7° C				At 20° C			
	MPN/100 g			Plate Count per g	MPN/100 g			Plate count per g
	Coliform	Salmonella	E. coli		Coliform	Salmonella	E. coli	
0	23,000	4,900	220	8,800	23,000	4,900	220	8,800
1/3					95,000	7,900	1,700	35,000
1	33,000	11,000	220	50,000	49,000,000	490,000	330,000	9,400,000
2								
3-1/2	790,000	7,000	310	500,000				
6	3,300,000	4,600	140	5,300,000				

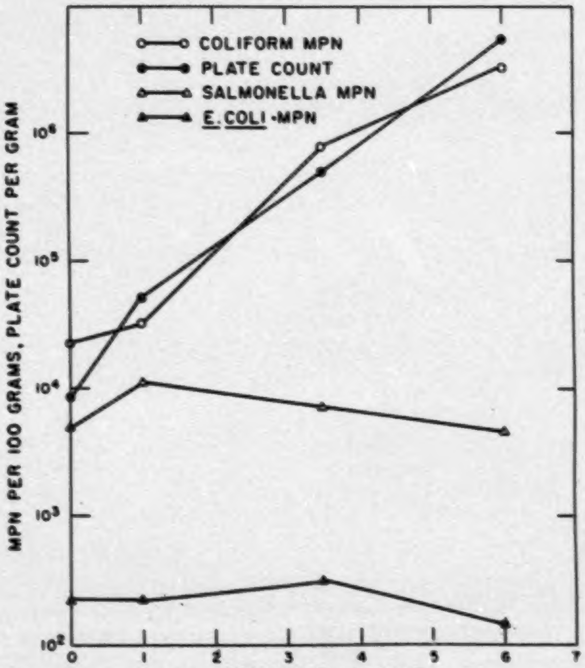

Figure 22. E. coli salmonella, and coliforms in shucked oysters stored at 4° to 7° C

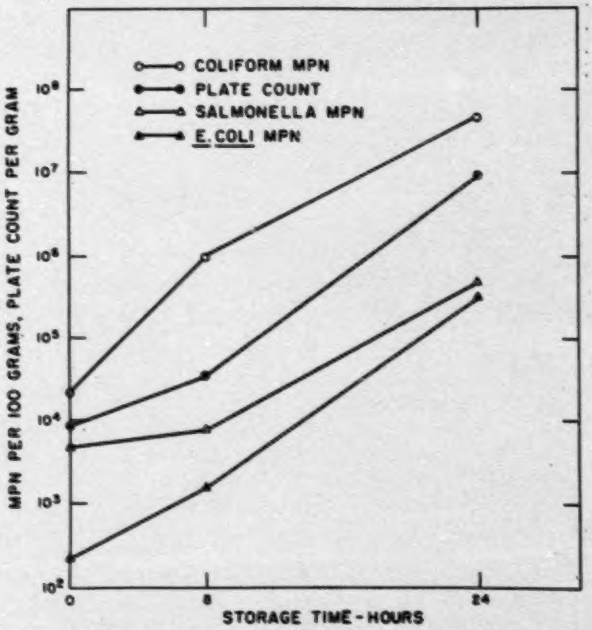

Figure 23. E. coli, salmonella, and coliforms in shucked oysters stored at 20°C

RESULTS AND DISCUSSION

A summary of results represented by median values determined from the combined data from the seven experiments is presented in Table 13 and in Figures 22 and 23. The use of median values was necessitated by indeterminate MPN values obtained from some experiments. The results of the individual experiments on which these results are based are contained in the appendix.

At a storage temperature of 4° to 7°C median, *E. coli* and salmonella densities remained relatively unchanged throughout 6 days of storage. By contrast, the coliform content and plate count showed progressive and significant multiplication within 3-1/2 days of storage. In oysters stored at 20°C all bacterial types tested for had increased significantly within 24 hours.

The significant increases in the coliform content and plate count of shucked oysters stored at 4° to 7°C, in contrast with a relatively unchanging concentration of enteric pathogens, would tend to negate the usefulness of the two former groups of organisms in assessing the sanitary quality of market oysters. Conversely, the parallel-behavior of *E. coli* and salmonella both at 4° to 7°C and at 20°C supports the assumption that *E. coli* and enteric pathogens would show a similar pattern of change and adds more evidence to justify the 1958 workshop selection of *E. coli* as an index of the sanitary quality of eastern oysters at the market level.

PART IIB. EVALUATION OF METHODS FOR ENUMERATING *E. COLI*

SUMMARY

Four methods for the quantative enumeration of *E. coli* type I in samples of sea water, shell oyster washings, and oyster meat were evaluated. The methods included the Eijkman, the E C, and the BALB liquid confirmatory procedures and the LSL-IMViC completed test.

Neither the Eijkman method nor the LSL-IMViC completed test was considered satisfactory, inasmuch as both methods showed a low degree of specificity for *E. coli* type I and permitted excessive growth of non-coliform and other coliform types.

Both the E C and BALB procedure demonstrated a relatively high degree of specificity for *E. coli* type I; however, the BALB method produced a higher percentage of false negative results, indicating that this procedure was more inhibitory to certain strains of *E. coli* than the E C method.

The E C method was considered the procedure of choice, and an outline of the proposed test was submitted to and accepted by agencies participating in a cooperative study to evaluate the relative merits of the coliform group and *E. coli* as indicators of the sanitary quality of eastern oysters at the market level.

INTRODUCTION

In recent years the tendency has grown on the part of agencies responsible for the sanitary control of shellfish to question the significance of the entire group of coliform organisms in assessing the sanitary quality of market oysters. It was suggested at the 1956 Shellfish Sanitation Workshop[9] that *E. coli* might be a more meaningful indicator of fecal pollution and that the relative merits of this organism and the entire coliform group in assessing the sanitary quality of market oysters should be investigated.

One of the major objections to the use of *E. coli* as an indicator organism has been the lack of a simple, rapid, reliable method of enumeration. The three more promising methods for enumerating *E. coli* include the Eijkman,[15] the E C,[16] and the boric acid lactose broth[17] (BALB) liquid confirmatory procedures. All three methods rely primarily on the ability of *E. coli* to grow and produce gas, in selected media, at temperatures higher than those tolerated by other coliform types. None of the three methods has been evaluated sufficiently to warrant its acceptance as a standard method for determining quantitatively *E. coli*. Consequently, before beginning participation in a cooperative study to determine the relative merits of *E. coli* and the entire coliform group in assessing the sanitary quality of market oysters, the Shellfish Sanitation Laboratory evaluated the reliability of the three above methods for enumerating this organism. For the purpose of comparing results from a method that did not rely on a selective temperature, an evaluation of the LSL-IMViC completed test also was included.

MATERIALS AND METHODS

The relative reliability of the three confirmatory methods in isolating *E. coli* was evaluated by submitting coliform cultures from all gas positive presumptive tubes from a number of samples of water, shell oyster washings, and oysters to a simultaneous examination by two of these procedures. Cultures from the gas positive presumptive tubes were transferred to the confirmatory media and incubated in water baths. Incubation temperatures were: Eijkman broth, 44° ± 0.2°C; BALB, 43° ± 0.2°C; E C medium, 44.5° ± 0.2°C. Cultures from all confirmatory tubes showing the presence of gas within 24 or 48 hours were streaked on EMB agar and incubated for 18 to 24 hours at 35° ± 0.5°C. If all colonies on an EMB plate were identical in colonial appearance, only one colony was selected for transfer to lactose broth. If colonies differed in appearance, one of each type was transferred. The lactose broth tubes were incubated in an air incubator at 35° ± 0.5°C. All cultures producing gas within 24 or 48 hours were submitted to the IMViC test. Cultures were classed according to the following IMViC reactions: ++-- *E. coli* type I, --++ *A. aerogenes* type II, -+-+ *Intermediate* type I, ++-+ *Intermediate* type II. Other IMViC reactions were recorded as observed.

An evaluation of the LSL-IMViC method was made by streaking cultures from all gas positive LSL presumptive tubes on EMB agar and following the procedure outlined for the liquid confirmatory methods.

The relative reliability of a method was based on the percentage of false-positive and false-negative confirmatory tubes produced by that method. A false-positive tube was one producing gas from which *E. coli* type I was not isolated. A false-negative *tube* was either a gas-positive or negative tube, by a given method, from which *E. coli* type I was not isolated, although the *corresponding* tube from one or both of the other methods showed the presence of *E. coli* type I.

RESULTS

Since the time and material involved did not permit the simultaneous examination of samples by all four methods, the LSL-IMViC completed test and the Eijkman and BALB liquid confirmatory methods were compared in the first series of experiments. A total of 339 gas-positive, presumptive tubes from 17 samples of oysters and 10 samples of shell oyster washings were examined by the three methods. The results of these experiments are presented in Table 14.

Based on the number of gas positive confirmatory tubes, BALB showed a high degree of specificity for *E. coli* type I. Approximately 92 percent of the *BALB gas* positive tubes contained *E. coli* type I, and only 8 percent were falsely *positive*. By comparison, the Eijkman procedure showed a low degree of specificity, with approximately 62 percent of the gas positive tubes yielding *E. coli* type I and approximately 39 percent *falsely positive* for this type. The two methods gave comparable results, with approximately 10 percent of the Eijkman tubes and about 13 percent of the BALB tubes falsely negative for *E. coli* type I. Based on the total number of LSL *presumptive* tubes demonstrated to contain *E. coli* type I by any of the methods followed, *the LSL-IMViC* completed test showed an extremely low degree of recovery. Out of a total of 78 tubes demonstrated to contain *E. coli* type I, the LSL-completed IMViC method *showed* the presence of these organisms in only 28 (36 percent) of the tubes.

Based on the above results, the BALB procedure was considered to be the most reliable of the three methods and, therefore, was compared with the E C procedure in a second series of experiments. In this series a total of approximately 1100 gas positive presumptive tubes from 13 water, 5 shell washings, and 88 oyster samples were examined by the E C and BALB methods. Although the LSL-IMViC completed test was demonstrated to be relatively unreliable in isolating *E. coli*, the same number of gas positive *presumptive tubes* were examined by this method to increase the probability of isolating *E. coli* type I from the presumptive tubes. The *results* of this second series of experiments are shown in Table 15.

The two methods of enumeration showed entirely different characteristics, with the E C method producing a higher percentage of false positive tubes and the BALB procedure a higher percentage of false negative tubes. Based on a total of 275 gas positive presumptive tubes demonstrated to contain *E. coli* type I by a combination of the three *methods, 93.4* percent of the tubes were shown to contain *E. coli* type I by the E C method and 82.5 percent *by the* BALB procedure. On the same basis of comparison the percentage of BALB tubes falsely negative (13.4 percent) was approximately twice as great as the percentage of E C tubes (6.5 percent) in this category. As in the first series of experiments, the percentage of presumptive tubes

TABLE 14. COMPARATIVE ISOLATIONS OF E. COLI TYPE I BY THE EIJKMAN AND BALB LIQUID CONFIRMATORY METHODS

	Eijkman		Balb	
	Number	Percent	Number	Percent
Tubes examined	339		339	
Tubes gas positive	114	33.6	74	23.3
Tubes E. coli type I positive	70	61.5	68	91.9
Tubes false positive[a]	44	38.8	6	8.1
Total LSL presumptive tubes E. coli type I positive[b]	78		78	
Confirmatory tubes E. coli type I positive [c]	70	89.7	68	87.2
Confirmatory tubes false negative[c]	8	10.3	10	12.8
LSL tubes E. coli type I positive via LSL[c]	28	36		

[a] Percent based on number of tubes gas positive.

[b] Based on number of presumptive tubes demonstrated to contain E. coli type I by any of the methods followed.

[c] Based on total LSL tubes E. coli type I positive.

TABLE 15. COMPARATIVE ISOLATIONS OF E. COLI TYPE I BY THE E C AND BALB LIQUID CONFIRMATORY METHODS

	E C		BALB	
	Number	Percent	Number	Percent
Tubes examined	1116		1112	
Tubes gas positive	291		242	
Tubes E. coli type I positive	257	88.3	238	98.3
Tubes false positive[a]	34	11.6	4	1.6
Total LSL presumptive tubes E. coli type I positive[b]	275		275	
Confirmatory tubes E. coli type I positive [c]	257	93.4	238	86.5
Confirmatory tubes false negative[c]	18	6.5	37	13.4
LSL tubes E. coli type I positive via LSL[c]	133	47.4		

[a] Percent based on number of tubes gas positive.

[b] Based on number of presumptive tubes demonstrated to contain E. coli type I by any of the methods followed.

[c] Based on total LSL tubes E. coli type I positive.

TABLE 16. INCIDENCE OF IMVIC REACTIONS OF CULTURES FROM GAS POSITIVE EIJKMAN, BALB, AND E C CONFIRMATORY TUBES

IMVIC reactions	Eijkman		BALB		E C	
	No.	Percent	No.	Percent	No.	Percent
Total cultures isolated	108		484		541	
E. coli I ++--	73	67.6	462	95.4	493	91.1
E. coli II -+--	5	4.6	6	1.2	2	0.37
A. aerogenes I --++	8	7.4	8	1.7	38	7
A. aerogenes II +-++	3	2.8	0		1	0.18
Intermediate I -+-+	13	12	0		0	
Intermediate II ++-+	4	3.7	4	0.8	0	
++++	0		1	0.2	0	
-+++	0		1	0.2	1	0.18
-++-	0		0		1	0.18
---+	0		2	0.4	0	
other[a]	12	11.1	0		5	0.9

[a] Gas-producing non-coliform organisms.

demonstrated to contain E. coli type I by the LSL-IMViC test was extremely low (47.4 percent), again demonstrating the unreliability of this method in isolating this organism.

Table 16 presents data on the coliform types isolated from the gas positive liquid confirmatory tubes.

The E C and BALB methods showed a relatively high degree of specificity for E. coli type I, with more than 90 percent of the total isolates giving a ++-- IMViC reaction. The next most frequently encountered reaction was that produced by A. aerogenes type I(--++). The percentage of --++ reactions was much greater from the E C cultures (7 percent), however, than from the BALB isolates (1.7 percent). The Eijkman procedure showed a relatively low degree of specificity, with only about 68 percent of the cultures producing E. coli type I reactions. Twelve percent of the cultures were Intermediate type I (-+-+) organisms, and approximately 10 percent of the cultures belonged in the A. aerogenes group. Approximately 11 percent of the cultures were classed as non-coliform organisms, on the basis of appearance on EMB agar and failure to produce gas in lactose broth after 24 or 48 hours of incubation at 35° ± 0.5°C.

DISCUSSION

Neither the Eijkman procedure nor the LSL-IMViC completed test was considered satisfactory for enumerating E. coli type I. Both methods showed a relatively low degree of specificity for this type organism and both permitted an excessive growth of other coliform and non-coliform types. The LSL-IMViC test was particularly unsatisfactory, inasmuch as the EMB agar permitted the growth of all coliform types. Since the other coliform types were much more numerous than E. coli type I and the EMB agar afforded no method of differentiation, the probability of selecting E. coli type I from the plates was greatly reduced.

Although the BALB and E C methods showed a comparable degree of specificity for E. coli type I, the BALB procedure showed a lower recovery rate and a greater percentage of false negative results, indicating that the method was more inhibitory for certain strains of E. coli type I. Consequently, the E C method was considered the procedure of choice. An outline of the method was submitted to and accepted by the agencies perticipating in a cooperative study to evaluate the relative merits of the coliform group and E. coli as indicators of the sanitary quality of market oysters.

Relationships Between E C Completed and E C Partially Confirmed MPN's

The use of the E C completed test, involving differentiation of the coliform group by their IMViC reactions, was necessary in evaluating the reliability of the E C method in enumerating E. coli type I organisms. The completed test is so demanding of time and material, however, that the procedure could not be routinely performed in many laboratories. Furthermore, the minimum time of 10 days required for completion of the test makes the method impractical for assessing the sanitary quality of a readily perishable product such as oysters. If the E C partially confirmed (E C gas positive) test could be applied with a satisfactory degree of confidence, however, results would be available in most instances within 48 hours, with no more expenditure of time and material than that required for the routine multitube confirmatory test for coliform organisms.

A comparison of E C completed and E C partially confirmed MPN's obtained from 81 Gulf Coast oyster samples collected at various stages of harvesting and processing shows a very close correlation between the MPN's, with a maximum variation of one tube at all levels (Table 17). By the very nature of the methods, the partially confirmed test always produces MPN's equal to or greater than those from the completed test. Thus, the former method would tend to provide a greater margin of public health safety.

Data from a cooperative study of eastern oysters[18] at the market level, collected primarily from the Middle Atlantic region, reveals a close relationship between the E C completed and E C partially confirmed results. On the basis of these data, the 1958 Shellfish Sanitation Workshop[14] recommended the adoption of a new interim standard for eastern oysters at the market level. The new standard was based on the partially confirmed E C test as an index of fecal pollution. Our results, although similar to the above data, show an even closer correlation of MPN's from the partially confirmed and completed test, thus indicating the validity of application of the E C partially confirmed test to eastern oysters from the Gulf Coast region.

TABLE 17. COMPARISON OF E C GAS POSITIVE AND *E. COLI* TYPE I MPN'S – NUMBER OF SAMPLES IN STATED GROUP

E C Gas Positive MPN'S	E. coli Type I MPN											
	18	20	45	78	130	230	330	490	790	1,300	2,400	Totals
18	14											14
20	2	13										15
45		6	6									12
78			4	7								11
130				5	7							12
230					3	1						4
330						2	0					2
490							2	1				3
790								2	1			3
1,300									2	2		4
2,400											1	1
Totals	16	19	10	12	10	3	2	3	3	2	1	81

REFERENCES

1. Kelly, C. B., Arcisz, W., Presnell, M. W., and Harris, E. K., Bacterial Accumulation by the Oyster Crassostrea virginica on the Gulf Coast. Sanitary Engineering Center Technical Report No. F60-4 U. S. Pub. Health Service (1959).

2. Knudsen, M., The Determination of Chlorinity by the Knudsen Method. Woods Hole Oceanographic Institution (1946).

3. Recommended Procedures for the Bacteriological Examination of Shellfish and Shellfish Waters. Am. J. Pub. Health 37 1121-1127 (1947).

4. Velz, C. J., Graphical Approach to Statistics: IV Evaluation of Bacterial Densities. Water and Sewage Works 98, 66-73 (1951).

5. Sanitary Control of the Shellfish Industry: Manual of Recommended Practice Part I: Growing Areas. U. S. Dept. of Health, Education & Welfare, Publ. Health Service (1959).

6. Manual of Recommended Practice for Sanitary Control of the Shellfish Industry. Federal Security Agency, Pub. Health Service, PHS Pub. No. 33, 12 (1946).

7. Wilson, Thomas E. and McClesky, C. S., Indices of Pollution in Louisiana Oysters. Food Research 16, 313 (1951).

8. Kelly, C. B., Bacteriological Examination as an Indicator of Sanitary Quality of Market Shellfish. Proceedings Shellfish Sanitation Workshop, U.S. Dept. of Health, Education & Welfare, Pub. Health Service (1956).

9. Proceedings Shellfish Sanitation Workshop. U. S. Dept. of Health, Education & Welfare, Pub. Health Service (1956).

10. Loosanoff, U. S., Behavior of Oysters in Water of Low Salinities. Proceedings Nat. Shellfisheries Assoc. 135-151 (1952).

11. Standard Methods for the Examination of Water, Sewage and Industrial Wastes, Tenth Edition. Am. Pub. Health Assoc. 1955 New York, N. Y.

12. Standard Methods for the Examination of Dairy Products, Tenth Edition, Am. Pub. Health Assoc., New York (1948).

13. Hauser, G. H., Bacteriological Quality of Market Oysters in Louisiana. Proceedings 1958 Shellfish Sanitation Workshop. Washington, D. C. (1958).

14. Proceeding Shellfish Sanitation Workshop. U. S. Dept. of Health, Education & Welfare, Public Health Service. (1958)

15. Eijkman, C., Die Gärungsprobe bei 46°C. Als Hilfsmittel bei der Trinkwasseruntersuchung. Centr. Bakt., II Abt., 37, 742 (1904).

16. Hajna, A. A. and Perry, C. A., Comparative Study of Presumptive and Confirmatory Media for Bacteria of the Coliform Group and for Fecal Streptococci. Am. J. Pub. Health, 3 550-556 (1943).

17. Vaughn, R. H., Levine, M., and Smith, H. A., A Buffered Boric Acid Lactose Medium for Enrichment and Presumptive Identification of Escherichia coli. Food Research 16, 10-19 (1951).

18. Kelly, C. B., Bacteriological Criteria for Market Oysters. Sanitary Engineering Center Technical Report No. F60-2. U. S. Public Health Service (1960).

APPENDIX

TABLE A1. BACTERIOLOGICAL STUDY OF OYSTER HARVESTING IN AREA A, STATION 1 - NORTH OF GORRIE BRIDGE

Date	Time	Water					Bottom Deposit			Shell Oysters
		Temperature, °C		Salinity, ppt		Coliform MPN per 100 ml	Settleable solids, %	Coliform MPN per 100 ml[a]	Volatile matter, %	Coliform MPN per 100 ml
		Top	Bottom	Top	Bottom					
6-13-56	7:30	27	28	17.2	17.6	49	8	2,100	0.34	
6-14-56	8:15	27	26	21.3	21.3	17	4	3,300	0.28	220
9-4-56	9:38	29	29	6.5	8.6	70				330
	16:43	30	30	18.4	19.8	7.8	9	2,400	1.96	220
9-19-56	9:06	28	27	19.5	19.9	920				310
	11:45	28	27	12.8	25.0	79				490
10-8-56	8:10	22	22	2.3	7.6	1,300				700
	15:40	26	25	2.2	3.3	170	7	1,100	1.66	3,300
11-8-56	10:45	21	22	13.4	25.1	64	9	900	1.70	78
	14:40	22	22	14.3	22.7	170				170
12-10-56	9:45	14	15	9.5	18.0	79	8	1,400	1.98	210
	13:55	16	15	3.4	13.0	46				460
1-8-57	7:40	11	12	<1.8	8.0	350				13,000
	14:30	14	14	<1.8	< 1.8	>1,600	13	10,000	2.10	490
2-5-57	15:55	23	23	<1.8	10.1	130	3	5,700	1.24	1,700
3-19-57	8:00	18	19	2.1	4.2	170		2,300	1.78	1,300
	11:30	19	19	8.2	4.6	170				790
9-17-57	8:11	28	28	3.0	3.3	790	7	3,100	1.30	790
	15:30	28	28	<1.8	<1.8	46				330
10-23-57	15:30	23	23	11.7	16	23	8	250	1.72	220

[a] Per 100 ml of settleable solids.

TABLE A2. BACTERIOLOGICAL STUDY OF OYSTER HARVESTING IN AREA A, STATION 2 - NORTH END NORMAN'S BAR

Date	Time	Water					Bottom Deposit			Shell Oysters
		Temperature, °C		Salinity, ppt		Coliform MPN per 100 ml	Settleable solids, %	Coliform MPN per 100 ml[a]	Volatile matter, %	Coliform MPN per 100 ml
		Top	Bottom	Top	Bottom					
6-25-56	8:55	28	27	20.3	24.0	240				1,300
	12:31	28	28	22.2	29.7	23	22	500	1.72	170
7-23-56	7:55	28	28	19.0	22.3	23				460
	11:30	31	30	19.7	22.7	33	8	980	1.48	230

[a] Per 100 ml of settleable solids.

TABLE A3. BACTERIOLOGICAL STUDY OF OYSTER HARVESTING IN AREA A, STATION 3 - SOUTH END NORMAN'S BAR

Date	Time	Water					Bottom Deposit			Shell Oysters
		Temperature, °C		Salinity, ppt		Coliform MPN per 100 ml	Settleable solids, %	Coliform MPN per 100 ml[a]	Volatile matter, %	Coliform MPN per 100 ml
		Top	Bottom	Top	Bottom					
6-25-56	7:47	28	28	20.3	29.4	79				230
	9:00									790
	11:50	28	27	29.7	30.6	22	7	1,900	0.47	130
7-23-56	7:40	28	28	18.9	24	17				130
	11:45	29	29	17.2	23.3	13	11	1,300	2.13	230
9-4-56	8:16	28	28	19.3	21.7	2.0				78
	14:45	30	31	19.3	20.5	2.0	13	150	1.88	20
9-19-56	7:40	26	26	24.9	28.7	7.8				330
	10:40	28	28	27.5	28.4	130				1,300
10-8-56	6:50	23	24	20.1	20.2	79				790
	13:30	25	24	4.0	12.0	240	11	2,500	2.04	790
11-8-56	8:00	21	22	26.7	30.2	< 1.8	5	< 360	1.65	45
	13:40	22	22	14.3	28.0	17				220
12-10-56	13:25	17	17	15.8	19.7	< 1.8	3	< 600	1.67	330
1-8-57	8:15	13	13	12.1	15.0	240				1,700
	11:15	14	14	14.4	14.4	130	20	1,200	2.21	2,300
2-5-57	14:30	23	23	6.6	13.0	7.8	14.5	< 130	1.99	310
3-19-57	8:35	18	19	5.8	11.6	49				270
	11:00	20	20	4.7	12.6	22	5.0	6,600	1.35	330
10-23-57	15:03	24	24	27.4	27.4	4.5				130

[a] Per 100 ml of settleable solids.

TABLE A4. BACTERIOLOGICAL STUDY OF OYSTER HARVESTING IN AREA A, STATION 4 - SOUTH END CATPOINT

Date	Time	Water					Bottom Deposit			Shell Oysters
		Temperature, °C		Salinity, ppt		Coliform MPN per 100 ml	Settleable solids, %	Coliform MPN per 100 ml[a]	Volatile matter, %	Coliform MPN per 100 ml
		Top	Bottom	Top	Bottom					
9-4-56	9:05	28	28	23.5	21.9	3.6				790
	15:45	30	30	27.5	27.5	<1.8	7	290	1.72	230
9-19-56	8:30	27	28	25.8	29.7	17				790
	11:20	29	28	25.0	27.8	13				790
10-8-56	7:25	25	25	27.2	28.6	13				230
	14:50	26	27	24.1	24.1	23	9	7,800	1.80	1,300
11-8-56	9:05	21	22	32.2	32.8	4.5	14	320	1.96	330
	14:05	22	22	30.0	30.4	17				130
12-10-56	10:25	15	15	25.6	25.6	<1.8	15	120	2.21	40
	12:25	17	16	25.5	26.6	<1.8				78
1-8-57	8:40	14	14	16.5	16.5	49				3,300
	10:10	13	14	18.9	18.3	79	11	180	1.96	3,100
2-5-57	15:05	23	23	17.2	18.8	4.5	10.5	180	1.79	410
3-19-57	9:15	20	20	13.4	16.6	33	7.0	1,100	1.52	170
	10:30	19	20	10.5	13.4	27				230
3-27-57	14:30	19	18	17.9	21.3	7.8				490
9-17-57	10:20	29	29	8.6	14.0	350	12	9,200	1.50	790
	14:50	29	29	8.6	19.4	79				790
10-23-57	14:40	23	23	27.2	1.8	<1.8	9	440	1.86	170

[a] Per 100 ml of settleable solids.

TABLE A5. BACTERIOLOGICAL STUDY OF OYSTER HARVESTING IN AREA A, STATION 5 - FERRY DOCK CATPOINT (NORTH END)

Date	Time	Water					Shell Oysters
		Temperature, °C		Salinity, ppt		Coliform MPN per 100 ml	Coliform MPN per 100 ml
		Top	Bottom	Top	Bottom		
9-4-56	9:14	29	29	23.4	23.4	4.5	230
	15:45	30.5	31	26.7	26.9	2.0	18
9-19-56	8:40	27	28	29.0	29.6	11	3,300
	11:25	28	27	27.5	28.2	2.0	330
10-8-56	7:38	24	24	28.5	28.2	4.5	7,000
	14:10	27	27	25.6	25.8	33	4,900
11-8-56	9:20	21	22	32.2	32.8	2.0	20
	14:20	22	22	30.2	30.8	<1.8	45
12-10-56	10:45	16	15	26.1	26.1	<1.8	68
	12:15	17	16	26.0	26.6	<1.8	220
1-8-57	9:07	14	14	16.2	17.3	350	7,900
	10:30	14	14	17	17	27	1,700
2-5-57	15:20	24	24	17.6	17.8	4.5	68
3-19-57	9:30	19	20	13.7	16.9	17	78
	10:45	19	20	10.5	11.8	79	40
3-27-57	15:00	20	20	20.3	20.3	14	790
9-17-57	10:40	29	29	4.0	6.0	70	230
	14:40	29	29	12.9	19.4	130	1,300
10-23-57	14:25	24	24	27.4	27.1	70	3,300

TABLE A6. BACTERIOLOGICAL STUDY OF OYSTER HARVESTING IN AREA B, STATION 6 - SHELLBANK REEF

Date	Time	Water					Bottom Deposit			Shell Oysters
		Temperature, °C		Salinity, ppt		Coliform MPN per 100 ml	Settleable solids, %	Coliform MPN per 100 ml[a]	Volatile matter, %	Coliform MPN per 100 ml
		Top	Bottom	Top	Bottom					
2-27-56	11:30	19	19	8.9	8.9	< 1.8	29	70	0.72	
3-5-56	10:39	18	18	5.3	5.3	31	9	200	0.16	45
3-26-56	10:20	16	16	6.3	6.6	7.8	14	320	0.45	170
4-2-56	12:45	20	21	5.8	5.8	31	5	900	0.13	120
4-17-56	10:30	20	20	3.9	4.7	33				
	10:58	20	20	4.2	4.4	9.2	17	460	0.31	9,500
4-30-56	9:35	25	25	2.6	3.4	11				
	11:15	24	24	2.6	3.4	23	16	430	0.38	330
5-21-56	9:50	28	28	6.7	6.5	< 1.8				
	11:40	27	27	5.9	6.2	4.5	20	100	0.23	20
6-5-56	10:20	24	24	12.1	12.1	4.5	12	170	0.27	20
7-2-56	9:45	30	30	13.0	13.1	< 1.8	18	110	1.37	330
7-30-56	12:30	30	30	16.2	15.7	2.0	13	850	0.61	20
8-13-56	9:54	30	30	13.7	15.7	< 1.8	18	< 100	1.51	790
9-14-56	10:40	28	28	22.5	23.0	4.5	10	610	1.36	20
10-2-56	10:58	25	26	21.1	22.0	< 1.8	14	290	1.43	45
11-28-56	10:40	10	10	20.7	20.7	< 1.8	19	360	1.36	170
12-4-56	11:00	15	15	19.3	23.0	< 1.8	16	110	1.38	18
2-26-57	9:47	17	17	12.1	12.7	33	8	2,800	1.22	68
6-19-57	10:30	31	30	8.7	10.1	1.8	14	320	1.40	130
8-21-57	10:35	29	29	20.7	21.0	< 1.8	11	< 160	1.66	45

[a] Per 100 ml of settleable solids.

TABLE A7. BACTERIOLOGICAL STUDY OF OYSTER HARVESTING IN AREA B, STATION 7 -NELSON'S REEF

Date	Time	Water					Bottom Deposit			Shell Oysters
		Temperature, °C		Salinity, ppt		Coliform MPN per 100 ml	Settleable solids, %	Coliform MPN per 100 ml[a]	Volatile matter, %	Coliform MPN per 100 ml
		Top	Bottom	Top	Bottom					
2-27-56	10:00	19	19	8.4	8.4	4.5	18	< 100	0.33	
3-5-56	9:47	18	18	2.6	2.8	33	15	1,500	0.26	61
3-26-56	9:25	16	16	6.0	6.0	49	9	1,900	0.18	68
4-2-56	11:25	21	21	5.0	5.0	7.8	10	450	0.19	490
4-17-56	10:20	21	21	3.1	3.4	17				
	10:55	21	21	3.1	3.4	46	14	930	0.28	400
4-30-56	9:15	25	24	1.8	1.8	14				
	10:30	25	25	1.8	2.6	17	18	1,300	0.38	93
5-21-56	9:20	28	28	6.2	6.7	4.5				
	12:12	28	28	7.8	7.0	4.5	9	500	0.21	230
6-5-56	9:35	24	24	9.2	9.2	4.5	13	350	0.22	< 18
7-2-56	10:30	30	28	11.7	12.2	6.8	24	80	1.11	230
7-30-56	11:30	29	29	11.6	12.7	7.8	4	500	1.16	490
8-13-56	9:12	31	30	12.9	13.5	4.5	22	350	1.35	1,300
9-14156	10.12	28	28	21.4	21.4	4.5	11	180	1.64	230
10-2-56	10.10	26	26	19.8	22.0	9.3	12	560	1.47	460
11-28-56	9:50	10	10	22.0	22.0	< 1.8	11	410	2.3	6.8
2-26-57	9:10	18	18	5.8	7.4	79	18	720	1.36	490
6-19-57	11:35	32	31	10.2	9.3	2.0	12	560	1.46	< 18
8-21-57	9:45	29.5	29	18.5	18.3	< 1.8	6	330	1.49	170

[a] Per 100 ml of settleable solids.

TABLE A8. BACTERIOLOGICAL STUDY OF OYSTER HARVESTING IN AREA B, STATION 8 - BON SECOUR REEF

Date	Time	Water					Bottom Deposit			Shell Oysters
		Temperature, °C		Salinity, ppt		Coliform MPN per 100 ml	Settleable solids, %	Coliform MPN per 100 ml[a]	Volatile matter,%	Coliform MPN per 100 ml
		Top	Bottom	Top	Bottom					
2-27-56	9:15	19	19	9.6	9.2	4.5				
	12:35	20	20	9.5	9.5	1.8	33	60	0.70	
3-5-56	9:27	18	18	8.8	7.6	9.3				
	11:35	19	18	7.9	7.9	2.0	28	500	0.55	230
3-26-56	9:05	16	16	6.8	6.0	33				
4-2-56	11:05	20	21	6.8	6.8	13				
	13:35	20	21	6.6	6.6	6.1	8	850	0.14	54,000
4-17-56	10:45	21	21	3.4	3.6	14	24	880	0.42	20
4-30-56	9:08	25	23	2.6	5.3	17				
	11:55	23	24	2.6	6.6	13	13	1,300	0.26	7,000
5-15-56	9:00	27	26	7.0	7.5	17				
5-21-56	9.03	28	28	8.9	8.3	4.5				
	12:55	28	28	9.1	8.6	4.0	8.5	810	0.22	40
6-5-56	9:10	25	25	11.6	11.9	11				
	11:07	25	25	11.9	11.9	<1.8	22	82	0.42	40
7-2-56	9:12	30	29	10.4	11.3	17				
	10:55	31	30	11.6	11.2	4.5	17	110	0.97	490
7-30-56	10:45	30	30	12.0	12.5	4.5				
	13:20	31	30	12.2	12.0	2.0	7	290	1.06	40
8-13-56	9:00	31	31	12.3	12.3	7.8				
	10:35	31	30	12.6	12.2	4.5	12	170	1.46	110
9-14-56	9:55	28	28	21.9	21.9	2.0				
	11:08	28	28	21.7	21.7	< 1.8				1,700
10-2-56	9:40	25	25	17.9	18.7	33				1,300
	11:56	25	26	18.2	21.2	130	12	1,900	1.24	
11-28-56	9:15	9	9	21.9	21.9	< 1.8				
	11:40	12	12	21.7	22.1	< 1.8	10	930	1.53	78
2-26-57	8:50	17	17	11.6	11.6	23				
	9:47	17	17	12.1	12.7	33	8	230	1.22	490
6-19-57	12:00	32	30	9.3	10.2	< 1.8	11	180	1.23	20
8-21-57	10:35	29	29	20.7	21.0	< 1.8	16	160	1.56	25

[a] Per 100 ml of settleable solids.

TABLE A9. BACTERIOLOGICAL STUDY OF OYSTER HARVESTING IN AREA M

Date	Time	Water					Shell Oysters
		Temperature, °C		Salinity, ppt		Coliform MPN per 100 ml	Coliform MPN per 100 ml
		Top	Bottom	Top	Bottom		
Half Moon Reef - Station 9							
11-5-57	11:30			12.4	12.4	2.0	170
11-20-57	11:05	18	18	5.3	5.3	110	4,900
12-10-57	11:03	12	12	1.8	1.8	130	110
	11:45	12	12	1.8	1.8	130	460
1-4-58	10:35	11	11	2.5	2.3	170	320
2-12-58	10:40	8	8	4.3	4.9	70	1.700
3-18-58	10:42	14	14	< 1.8	<1.8	79	170
4-2-58	10:38	18	18	< 1.8	<1.8	79	400
4-29-58	11:10	26	26	6.5	6.5	79	140
St. Joe Channel Reef - Station 12							
12-10-57	11:30	12	12	1.8	1.8	130	490
1-14-58	10:00	11	11	<1.8	<1.8	>2,300	790
	11:08	11	11	<1.8	<1.8	1,600	3,300
2-12-58	11:10	8	8	3.8	5.1	2,300	320
3-18-58	10:15	15	15	<1.8	<1.8	920	17,000
	12:55	14	14	<1.8	<1.8	920	790
4-2-58	10:21	18	18	<1.8	<1.8	920	2,200
	12:22	19	19	<1.8	<1.8	1,600	35,000
4-29-58	10:20	26	26	4.6	4.6	220	310
	12:40	26	26	4.6	5.2	22	230

TABLE A10. BACTERIOLOGICAL STUDY OF OYSTER HARVESTING IN AREA M

Date	Time	Water					Shell Oysters
		Temperature, °C		Salinity, ppt		Coliform MPN per 100 ml	Coliform MPN per 100 ml
		Top	Bottom	Top	Bottom		
Grassy Island Reef - Station 10							
11-5-57	11:00			12.4	12.4	2.0	270
	11:55			12.4	12.4	< 1.8	170
11-20-57	10:55	18	18	5.3	5.6	1,300	7,900
	11:55	18	18	5.6	5.8	79	3,300
12-10-57	10:48	12	12	1.8	1.8	33	490
	11:53	12	12	< 1.8	< 1.8	110	110
1-14-58	10:20	11	11	2.5	2.5	240	68
	10:45	11	11	2.0	2.0	79	22,000
2-12-58	10:20	8	8	6.1	5.9	49	1,700
	10:50	8	8	5.1	5.4	49	1,100
3-18-58	10:50	15	15	<1.8	< 1.8	240	54,000
	12:10	14	14	<1.8	<1.8	79	3,300
4-2-58	10:46	18	18	<1.8	<1.8	110	13,000
	12:05	19	19	<1.8	<1.8	350	400
4-29-58	10:58	26	26	6.0	6.0	240	490
	12:20	26	26	6.6	6.7	70	460

TABLE A11. BACTERIOLOGICAL STUDY OF OYSTER HARVESTING IN AREA M

Date	Time	Water					Shell Oysters
		Temperature, °C		Salinity, ppt		Coliform MPN per 100 ml	Coliform MPN per 100 ml
		Top	Bottom	Top	Bottom		
St. Joe Reef - Station 11							
11-5-57	10:15			12.4	17.8	4.5	2,200
	12:30			12.4	15.1	11	130
11-20-57	10:22	18	18	5.5	5.5	350	1,700
	12:30	18	18	5.5	5.3	280	1,300
12-10-57	10:15	12	12	<1.8	< 1.8	79	2,200
	12:18	12	12	<1.8	< 1.8	240	2,300
1-14-58	09:45	10	11	<1.8	<1.8	1,600	3,900
	11:30	11	11	<1.8	<1.8	> 2,300	11,000
2-12-58	09:50	8	8	6.6	7.4	920	2,400
	11:30	8	8	7.1	6.9	350	700
3-18-58	09:30	14	14	<1.8	<1.8	350	>160,000
	13:15	16	16	<1.8	<1.8	130	<18
4-2-58	09:47	18	18	<1.8	<1.8	920	
	12:33	19	19	<1.8	<1.8	540	4,900
4-29-58	10:05	25	25	6.2	6.7	1,600	230
	12:50	26	26	6.2	6.7	110	55

TABLE A12. BACTERIOLOGICAL STUDY OF OYSTER HARVESTING IN AREA P

Date	Time	Water					Shell Oysters
		Temperature, °C		Salinity, ppt		Coliform MPN per 100 ml	Coliform MPN per 100 ml
		Top	Bottom	Top	Bottom		
Pass Christian Reef - Station 13							
11-5-57	08:30			22.8	23.4	14	68
	14:00			23.1	23.1	17	1,300
11-20-57	08:35	18	18	14	14	170	1,300
	14:05	18	18	14	14	33	1,700
12-10-57	08:15	11	11	10.5	10.8	6.8	490
	13:45	13	12	7.7	7.6	79	490
1-14-58	08:10	10	10	7.6	7.6	540	490
	13:20	12	12	8.1	9.9	130	330
2-12-58	08:15	8	8	19	19	79	230
	13:10	8	8	19	19	4.5	330
3-18-58	08:15	14	14	3.9	3.9	79	230
	15:00	16	16	7.7	7.7	79	170
4-2-58	08:09	18	19	10.3	12.6	130	490
	14:30	20	20	12.9	13.4	13	1,400
4-29-58	08:25	25	25	8.6	8.6	34	1,400
	14:40	26	26	7.8	7.8	79	2,200

TABLE A13. COLIFORM MPN PER 100 GRAMS OF TRACED LOTS OF OYSTERS FROM AREA A

Date landed	Shell oyster washings			Shell oysters			Shucked oysters				
							In shuckers' pots			As packed	
	As dug	As landed	Before shucking	As dug	As landed	Before shucking	As shucked	Temperature, °C	MPN	Temperature, °C	MPN
6-13-56	92		140	< 18		< 18	3,300		490		330
6-25-56			490			45	230		78		130
6-25-56	490	24,000	54,000	750	1,300	3,300	3,300	28	1,700	26	2,300
6-25-56	220	1,300	3,300	600	130	230	230	26	790	25	7,900
7-23-56			490			2,900	2,300	27	2,300	28	1,100
7-23-56	18	160,000	160,000	180	2,300	4,900	4,600	27	2,200	25	790
7-23-56	45	2,300	4,900	400	330	330	230	26	330	28	950
9-4-56	20		1,100	230	790	2,300	230	25	490		1,700
9-4-56		790	1,300			78	310	25	700		790
9-4-56	1,300	5,600	13,000	430	220	1,100	3,300	25	13,000		4,900
9-19-56	18	460	170	1,800	330	700	700	26	1,300		1,700
9-19-56	20	330	400	790	330	230	1,700	26	490		790
9-19-56		45	20		770	490	330	26	1,300		2,300
10-8-56	>160,000	24,000	24,000	6,000	4,900	2,300	24,000		4,900		11,000
10-8-56		3,300	7,900		490	2,300	330		1,300		1,700
10-8-56	130		24,000	770	460	3,300	2,300	21	2,200		3,300
11-8-56	37	790	130	40	220	490	330	10	110		20
11-8-56	37	490	4,900	40	220	790	490	13	1,700		490
11-8-56	230	230	110	230	230	330	230	9	230		170
12-10-56		140	220		45	40	20	12	< 18	15	78
12-10-56	220	170	330	59	37	45	700	13	460	15	330
12-10-56	< 18	2,300	700	140	45	170	130	14	330	15	330
1-8-57	790	790	2,300	4,900	1,100	1,300	3,300		2,300		2,300
1-8-57		1,700	2,300		3,300	3,300	4,900		2,200		3,300
1-8-57	790	490	2,200	4,900	3,300	3,300	2,300		1,700		35,000
2-5-57		3,300	4,900		1,300	7,900	3,300	18	790	20	790
2-5-57	460	1,400	210	410	220	330	230	18	54,000	20	330
2-5-57	78	13,000	13,000	68	1,100	2,300	54,000	19	13,000	20	4,900
3-19-57	130	78	7,900	200	170	4,900	490		2,300		24,000
3-19-57	130	3,300	790	200	1,400	2,800	950		2,300		1,300
3-19-57	45	110	400	58	950	11,000	17,000		2,200		3,300
3-27-57					1,300	18	490				1,800
3-27-57	790	2,300	2,800	490	1,800	1,300	13,000		3,300		2,300
3-27-57	490	4,900	13,000	790	2,200	11,000	11,000		24,000		11,000
3-27-57					1,300	18	490				1,800
9-17-57	13,000	54,000	>160,000	700	1,800	7,900	14,000		11,000		4,900
9-17-57	13,000		54,000	540		35,000	3,300		24,000		17,000
9-17-57	490	13,000	28,000	790	330	490	2,300		1,300		4,900
10-23-57	78	110	790	3,300	20	490	1,300		700		4,900
10-23-57	1,700	310	2,300	170	4,900	3,300	1,300		490		1,100
10-23-57	490	4,300	14,000	130	270	3,300	4,900		3,300		1,300
2-4-58		7,900	92,000		2,300	4,900	7,900				3,300
2-4-58		490	490		3,300	700	2,200		2,200		1,100
2-4-58		22,000	280		2,300	490	1,300		4,900		700

TABLE A14. PLATE COUNT PER GRAM OF TRACED LOTS OF OYSTERS FROM AREA A

Date landed	Shell oyster washings			Shell oysters			Shucked oysters				
							In shuckers' pots			As packed	
	As dug	As landed	Before shucking	As dug	As landed	Before shucking	As shucked	Temperature, °C	Count	Temperature, °C	Count
6-13-56	3,100		26,000	400		900	23,000		5,900		6,500
6-25-56			33,000			600	23,000		4,100		3,400
6-25-56	2,500	68,000	600,000	800	1,700	9,900	13,000	28	27,000	28	48,000
6-25-56	5,200	5,800	24,000	1,500	600	1,600	3,000	26	1,700	25	43,000
7-23-56			49,000			1,000	2,800	27	3,500	28	700
7-23-56	1,000	24,000	43,000	700	1,600	2,300	11,000	27	12,000	25	83,000
7-23-56	1,900	6,900	4,900	1,400	2,700	1,900	14,000	26	12,000	28	5,800
9-4-56	2,100		140,000	1,600	600	3,300	10,000	25	4,500		4,500
9-4-56			150,000			5,000	4,200	25	13,000		12,000
9-4-56	12,000	8,200	120,000	1,100	1,000	1,500	12,000	25	3,300		9,200
9-19-56	2,000	13,000	33,000	1,000	700	1,000	5,800	26	2,300		11,000
9-19-56	1,900	2,700	4,400	1,900	700	600	4,100	26	800		2,500
9-19-56		1,600	1,700		600	1,100	700	26	1,600		23,000
10-8-56	36,000	2,200	67,000	10,000	1,000	1,800	8,000		3,800		3,900
10-8-56		24,000	51,000		600	1,300	2,400		4,400		32,000
10-8-56	7,700		10,000	71,000	1,000	1,000	3,800	21	4,800		10,000
11-8-56	3,300	2,500	1,000	1,300	900	1,200	5,100	10	3,000		8,800
11-8-56	3,300	1,600	2,800	1,300	900	900	400	13	700		1,400
11-8-56	1,600	2,100	19,000	1,100	1,600	3,100	900	9	700		16,000
12-10-56		3,000	2,800		1,100	800	1,100	12	800	15	2,700
12-10-56	16,000	3,500	7,500	2,200	300	800	2,100	13	900	15	17,000
12-10-56	4,300	5,300	11,000	5,400	400	1,200	7,900	14	2,000	15	3,300
1-8-57	3,700	3,400	9,300	3,000	1,000	1,400	2,300		2,700		3,200
1-8-57		6,400	6,100		1,500	2,500	22,000		3,100		3,000
1-8-57	3,700	3,900	8,500	3,000	1,700	2,400	4,600		2,200		35,000
2-5-57		2,400	6,400		1,300	2,000	2,500	18	2,200	20	4,600
2-5-57	2,300	1,700	2,300	1,300	700	1,100	500	18	2,200	20	9,600
2-5-57	1,700	16,000	13,000	1,000	900	1,500	2,900	19	4,300	20	11,000
3-19-57	2,600	500	170,000	1,300	1,100	12,000	7,200		6,100		9,500
3-19-57	2,600	5,600	21,000	1,300	800	4,500	5,700		6,400		2,600
3-19-57	2,100	9,700	27,000	700	600	5,000	30,000		14,000		16,000
3-27-57					700	300	3,200				12,000
3-27-57	11,000	2,200	7,200	2,900	1,600	1,000	3,500		1,700		2,200
3-27-57	5,000	1,400	26,000	1,500	1,800	4,400	19,000		4,600		1,700
3-27-57					700	200	3,200				12,000
9-17-57	15,000	61,000	92,000	1,000	1,900	6,100	23,000		130,000		6,900
9-17-57	15,000		TNC	1,000		28,000	53,000		37,000		26,000
9-17-57	2,300	24,000	110,000	800	800	3,600	18,000		12,000		47,000
10-23-57	6,800	2,500	22,000	2,100	1,400	1,700	5,300		2,500		7,000
10-23-57	13,000	12,000	24,000	3,600	2,100	2,000	16,000		9,000		4,800
10-23-57	5,100	31,000	50,000	1,100	1,700	3,400	27,000		30,000		20,000
2-4-58		3,900	20,000		12,000	5,100	4,200				6,400
2-4-58		35,000	35,000		2,900	4,700	3,100		4,400		18,000
2-4-58		13,000	28,000		4,300	3,700	4,400		3,700		25,000

TABLE A15. COLIFORM MPN PER 100 GRAMS OF TRACED LOTS OF OYSTERS FROM AREA B

Date landed	Shell oyster washings			Shell oysters			Shucked oysters				
							In shuckers' pots			As packed	
	As dug	As landed	Before shucking	As dug	As landed	Before shucking	As shucked	Temperature, °C	MPN	Temperature, °C	MPN
3-5-56			54,000	45		3,100	160,000	20	160,000		35,000
3-6-56			160,000			13,000	92,000	20	28,000		17,000
3-5-56			160,000	61		92,000	92,000	20	160,000		35,000
3-12-56		54,000	160,000		2,800	92,000	160,000	21	160,000		160,000
3-12-56			160,000			160,000[a]	54,000	21	160,000		17,000
3-21-56		3,100	680		180	180	4,000	14	49,000		3,300
3-21-56		1,100	2,300		180	1,700	13,000				
3-26-56	790	130	160,000	68	92	31,000	17,000	18	17,000		4,900
3-26-56			3,300			1,700[a]	74	18	560		7,900
4-3-56		1,400	160,000		92	220,000	240,000	23	240,000	23	14,000
4-3-56			160,000			35,000[a]	92,000	23	160,000	23	17,000
4-9-56		130	4,900		230	4,900	13,000	17	160,000	16	4,900
4-9-56		1,300	1,600,000		1,300	49,000	13,000	18	33,000	20	7,900
4-9-56			13,000			11,000[a]	11,000	18	23,000	20	3,300
4-17-56	220	17,000	160,000	9,500	170	17,000	54,000	15	17,000	19	1,700
4-17-56	2,300	24,000	160,000	460	1,700	7,900	35,000	18	17,000	21	17,000
4-17-56			160,000			13,000[a]	13,000	18	4,900	21	11,000
4-23-56		160,000	1,600,000		13,000	92,000	92,000	18	350,000	21	7,000
4-23-56			220,000			28,000[a]	160,000	18	170,000	21	22,000
4-30-56	1,300	54,000	540,000	93	1,100	17,000	22,000	26	920,000	24	35,000

[a] Same lot of shell oysters as on line above, but washed on landing with chlorinated water.

TABLE A15. (continued) COLIFORM MPN PER 100 MILLILITERS OF TRACED LOTS OF OYSTERS FROM AREA B

Date landed	Plant number	Shell oyster washings			Shell oysters			Shucked oysters				
								In shuckers' pots			As packed	
		As dug	As landed	Before shucking	As dug	As landed	Before shucking	As shucked	Temperature, °C	MPN	Temperature, °C	MPN
11-29-56	43		220	7,900		1,300	1,300	13,000	10	13,000	16	1,300
12-3-56	43			4,900			1,300	13,000	10	2,300	15	1,300
12-4-56	43	330	790	7,900	330	230	1,300	4,900	17	24,000	20	4,900
12-17-56	43	18	160,000	160,000	490	92,000	160,000	160,000	20	160,000	22	92,000
1-15-57	49		1,800	160,000		54,000	54,000	160,000	15	92,000	19	35,000
1-15-57	43		460	11,000		330	2,300	92,000	13	22,000	13	160,000
1-29-57	43		4,900	54,000		2,300	7,900	35,000	21	79,000	22	13,000
1-29-57[b]	49		31,000	92,000		2,300	7,900	11,000	22	23,000	22	4,900
3-13-57	43			92,000			3,300	130,000	19	7,900	20	160,000
3-13-57	36			13,000			14,000	33,000	19	11,000	20	3,300
3-13-57	49			92,000			17,000	7,900	19	23,000	21	4,900
1-28-58	36			2,200			1,100	4,600		1,700		2,300

[b] Oysters held 2 days.

TABLE A16. PLATE COUNT PER GRAM OF TRACED LOTS OF OYSTERS FROM AREA B

	Shell oyster washings			Shell oysters			Shucked oysters				
							In shuckers' pots			As packed	
Date landed	As dug	As landed	Before shucking	As dug	As landed	Before shucking	As shucked	Temperature, °C	Count	Temperature, °C	Count
3-5-56			250,000			8,000	42,000	20	35,000		17,000
3-6-56			860,000			19,000	110,000	20	67,000		26,000
3-5-56			510,000			66,000	250,000	20	180,000		39,000
3-12-56		21,000	1,600,000		5,300	290,000	640,000	21	510,000		71,000
3-12-56			430,000			300,000[a]	270,000	21	210,000		160,000
3-21-56		22,000	2,500		2,500	1,000	5,000	14	5,000		500
3-21-56		67,000	15,000		7,500	3,000	47,000				
3-26-56	5,500	12,000	300,000	2,000	3,000	64,000	82,000	18	94,000		17,000
3-26-56			110,000			30,000[a]	27,000	18	170,000		27,000
4-3-56		1,800	1,000,000		5,600	500,000	570,000	23	220,000	23	230,000
4-3-56			420,000			480,000[a]	710,000	22	440,000	23	84,000
4-9-56		2,700	69,000		3,200	19,000	91,000	17	67,000	18	7,400
4-9-56		5,000	280,000		19,000	237,000	77,000	18	130,000	20	42,000
4-9-56			65,000			160,000[a]	100,000	18	98,000	20	270,000
4-17-56	2,700	66,000	1,900,000	51,000	2,400	74,000	190,000	15	200,000	19	32,000
4-17-56	1,600	130,000	TNC	11,000	9,600	270,000	1,300,000	18	830,000	21	380,000
4-17-56			2,000,000			180,000[a]	1,100,000	18	260,000	21	110,000
4-23-56		290,000	6,200,000		45,000	290,000	400,000	18	530,000	21	54,000
4-23-56			2,000,000			220,000[a]	350,000	18	150,000	21	35,000
4-30-56	24,000	230,000	6,100,000	160,000	4,500	170,000	280,000	26	650.000	24	99,000
11-29-56		22,000	11,000		2,000	2,200	3,700	10	2,400	16	1,100
12-3-56			27,000			1,900	5,100	10	2,700	15	1,600
12-4-56	280,000	5,400	220,000	2,700	1,300	2,800	3,900	17	5,200	20	2,400
12-17-56	5,000	100,000	120,000	1,100	5,900	9,000	20,000	20	27,000	22	11,000
1-15-57		110,000	190,000		5,900	12,000	56,000	15	25,000	19	13,000
1-15-57		40,000	91,000		5,700	6,000	20,000	13	19,000	13	17,000
1-29-57		22,000	75,000		25,000	6,000	9,000	21	12,000	22	4,000
1-29-57		120,000	370,000		18,000	25,000	91,000	22	93,000	22	26,000
3-13-57		120,000	560,000			25,000	86,000		31,000		24,000
3-13-57			100,000			4,800	59,000		91,000		28,000
3-13-57			530,000			12,000	42,000		75,000		34,000
1-28-58			7,000			10,000	10,000		20,000		4,000

[a] Same lot of shell oysters as on line above, but washed on landing with chlorinated water.

TABLE A17. COLIFORM MPN PER 100 GRAMS OF TRACED LOTS OF OYSTERS FROM AREAS P AND M

Date landed	Shell oyster washings			Shell oysters			Shucked oysters		
	As dug	As landed	Before shucking	As dug	As landed	Before shucking	As shucked	In shuckers' pots	As packed
Area P									
11-5-57	40	4,900	3,300	300	170	2,300	35,000	13,000	3,300
11-20-57	78	4,900	17,000	1,500	3,300	4,900			13,000
	78		9,500	1,500		4,600	11,000	4,900	7,900
12-10-57	3,300	2,300	24,000	490	1,300	7,900	17,000	4,900	3,300
1-4-58	700		35,000	400	790	3,300	54,000	54,000	7,000
4-3-58	170		92,000	950		17,000	>160,000	35,000	28,000
Area M Mississippi Sound									
11-5-57		>160,000	>160,000	300[a]	>160,000	>160,000	>160,000	>160,000	>160,000
11-6-57		>160,000	>160,000	300[a]	54,000	>160,000	>160,000	>160,000	>160,000
1120-57		1,600,000	1,600,000	3,100[a]	540,000	1,600,000	1,700,000	170,000	240,000
11-21-57			110,000	3,100[a]		130,000	49,000	330,000	920,000
12-10-57			540,000	490[a]		49,000	540,000	540,000	49,000
12-10-57	1,400[b]	160,000	24,000	490[a]	3,300	35,000	160,000	160,000	35,000
1-14-57	230[b]		54,000	1,600		11,000	54,000	17,000	3,100
1-15-58			54,000			92,000	54,000	24,000	13,000
Louisiana									
2-13-58			>160,000			160,000	13,000	54,000	35,000
2-13-58			54,000			3,300	11,000	4,900	>160,000
2-13-58			11,000			7,900	54,000	33,000	
3-19-58			22,000			17,000	>160,000	92,000	22,000
3-19-58			>160,000			35,000	>160,000	54,000	>160,000
3-19-58			54,000			4,600	92,000	22,000	17,000
4-3158			>160,000			>160,000	>160,000	>160,000	>160,000
4-3-58			13,000			13,000	23,000	33,000	54,000
4-3-58			130,000			3,300	33,000	49,000	33,000

[a] Geometric mean of all samples except taken from area this date.
[b] Experimental lots.

TABLE A18. PLATE COUNT PER GRAM OF TRACED LOTS OF OYSTERS FROM AREAS P AND M

Date landed	Shell oyster washings			Shell oysters			Shucked oysters		
	As dug	As landed	Before shucking	As dug	As landed	Before shucking	As shucked	In shuckers' pots	As packed
Area P									
11-5-57	3,600	32,000	12,000	1,400	800	1,800	9,000	5,900	4,700
11-20-57	1,300	6,700	26,000	2,400	3,100	4,900			55,000
11-20-57	1,300		130,000	2,400		5,500	24,000	16,000	13,000
12-10-57	1,600	3,500	49,000	6,700	6,600	4,700	17,000	240,000	25,000
1-14-58	5,000		33,000	500	1,100	3,800	11,000	9,500	4,000
4-3-58	1,600		TNC	2,900		14,000	41,000	12,000	15,000
Area M Mississippi Sound									
11-5-57		TNC	TNC		TNC	250,000	670,000	530,000	600,000
11-6-57		TNC	TNC		410,000	TNC	TNC	TNC	TNC
11-20-57		2,400,000	4,800,000		220,000	180,000	810,000	660,000	290,000
11-21-57			3,700,000			330,000	780,000	820,000	260,000
12-10-27			2,100,000			140,000	490,000	250,000	59,000
12-10-57	19,000[a]	140,000	TNC	9,400	6,200	7,900	41,000	98,000	44,000
1-14-58	6,400[a]		73,000	21,000		11,000	42,000	29,000	4,300
1-15-58			41,000			2,000	17,000	10,000	4,500
Louisiana									
2-13-58			160,000			16,000	3,200	110,000	96,000
2-13-58			130,000			6,000	18,000	7,000	190,000
2-13-58			93,000			15,000	63,000	25,000	
3-19-58			28,000			TNC	TNC	TNC	TNC
3-19-58			TNC			11,000	63,000	60,000	40,000
3-19-58			TNC			18,000	81,000	45,000	36,000
4-3-58			420,000			63,000	81,000	140,000	130,000
4-3-58			89,000			33,000	47,000	14,000	12,000
4-3-58			65,000			14,000	26,000	60,000	19,000

[a] Experimental lots.

TABLE A19. EFFECT OF STORAGE ON BACTERIAL CONTENT OF OYSTERS FROM AREA A

Storage time, days	Storage temperature, °C	Coliform MPN/100 ml	E. coli MPN/100 ml	Plate count per ml	pH
Lot A1 - Shell oysters from North End Cat Point, dug 3-27-57 by Daniels Bros, Fla. 7, stored at room temperature.					
0	17-23	2,200		1,800	
1	19-28	24,000		8,800	
1-1/2	20-23	54,009		24,000	6.7
2	20	1,700		7,000	6.5
3	20-21	54,000		28,000	6.6
4	21-22	180,000		130,000	7.1
6		> 160,000		TNC	6.5
Lot A2 - Shell oysters same as lot A1 stored in refrigerator					
0		2,200		1,800	
1-1/2	2-8	3,300		3,200	6.7
4	2-9	35,000		27,000	6.8
8	2-10	79,000		130,000	7.0
11	3-8	170,000		44,000	6.8
Lot A3 - Shucked oysters dug at North End Cat Point 3-27-57, shucked 3-28-57 by Daniels Bros, Fla. 7, stored at room temperature.					
1/3	17-23	22,000		6,400	6.3
1	19-28	> 160,000		TNC	5.8
2	20-23	>16,000,000		TNC	4.9
Lot A4 - Shucked oysters same as Lot A3 stored in refrigerator.					
1/3		22,000		6,400	
1	2-8	24,000		14,000	6.4
2	1-4	7,900		40,000	6.2
3	2-9	13,000		6,000	6.2
4	4	17,000		9,900	6.2
5	3-11	24,000		38,000	5.9
6	2-10	22,000		15,000	5.9
8	4-6	7,900		26,000	5.9
9	4-6	23,000		1,300,000	5.9
10	3-8	17,000		50,000	5.9
11	3-10	70,000		71,000	5.8

TABLE A19. (continued) EFFECT OF STORAGE ON BACTERIAL CONTENT OF OYSTERS FROM AREA A

Storage time, days	Storage temperature, °C	Coliform MPN/100 ml	E. coli MPN/100 ml	Plate count per ml	pH
Lot A5 - Shucked oysters dug at North End Cat Point 9-18-57, shucked 9-19-57 by Daniels Bros., Fla. 7.					
0		4,900		6,900	6.4
1	4	24,000		1,500	6.4
2	5	4,900		8,900	
5	5	11,000		8,000	
7	3	2,200		12,000	6.1
9	4	4,600		11,000	6.1
12	2	2,500		4,600	6.0
14	2	7,900		9,100	6.1
19	3	13,000		7,400	6.0
21	2-5	7,000	130	4,600	5.9
23	2-5	17,000		5,300	5.9
Lot A6 - Shucked oysters dug at North End Cat Point 10-23-57, shucked 10-24-57 by Daniels Bros., Fla. 7.					
0		4,900		7,000	7.0
1	1.5	790		65,000	6.1
3	0	310		33,000	6.0
5	2	1,300		47,000	5.9
7	6	260		60,000	5.9
14	5	230		37,000	5.9
18	1	230		24,000	5.7
Lot A7 - Shucked oysters dug at North Gorrie Bridge 2-4-58, shucked 2-5-58 by Daniels Bros., Fla. 7.					
0		3,300	110	6,400	6.6
2	8	4,900		13,000	6.1
5	6	41,000		230,000	5.9
9	5	490,000	45	1,500,000	5.5
12	5	700,000	< 18	400,000	5.5
14	5.5	790,000		280,000	5.5
Lot A8 - Shucked oysters dug at North End Cat Point 2-4-57, shucked 2-5-57 by Daniels Bros., Fla. 7.					
0		1,100	78	18,000	6.6
2	8	7,000		18,000	6.1
5	6	28,000		6,000	5.9
9	5	840,000	40	4,400,000	5.6
12	5	330,000	20	1,000,000	5.4
15	6.5	330,000		740,000	5.5

TABLE A20. EFFECT OF STORAGE ON BACTERIAL CONTENT OF OYSTERS FROM AREA B

Storage time, days	Storage temperature, °C	Coliform MPN/100 ml	E. coli MPN/100 ml	Plate count per ml	pH
Lot B1 - Shell oysters tonged at Shellbank Reef 4-2-57, delivered to Bon Secour Fisheries, Ala. 49, 4-2-57, stored at room temperature.					
Shellstock		13,000		10,000	6.75
1	21-22	22,000		38,000	6.74
2	23-25	>1,600,000		590,000	6.71
3	18-23	>1,600,000		TNC	6.42
5	20-22	9,200,000		300,000	5.92
Lot B2 - Shell oysters same as lot B1, stored in refrigerator.					
Shellstock		13,000		10,000	6.75
2	2-12	33,000		12,000	6.92
6	2-9	33,000		82,000	6.85
Lot B3 - Shucked oysters tonged at Shellbank Reef 4-2-57, shucked 4-3-57 by Bon Secour Fisheries, Ala. 49, stored at room temperature.					
Shellstock		13,000		10,000	6.75
0		24,000		65,000	6.20
1	21-22	>1,600,000		2,000,000	5.88
Lot B4 - Shucked oysters same as lot B3, stored in refrigerator.					
Shellstock		13,000		10,000	6.75
0		24,000		65,000	6.20
1	2-10	23,000		63,000	6.08
2	2-12	49,000		57,000	6.34
3	4-6	49,000		90,000	5.88
4	4-6	13,000		71,000	5.79
5	4-10	23,000		51,000	6.08
Lot B5 - Shucked oysters tonged at Bon Secour Reef 1-27-58, shucked 1-28-58 by Fulford Fish & Oyster Co., Ala. 36, stored in refrigerator.					
Shellstock		1,100	40	10,000	6.9
0		2,300	40	4,000	6.9
1	7	3,300	<18		6.3
3	7	24,000		38,000	6.0
7	6	330,000	20	970,000	5.8
9	9	4,900,000		3,800,000	5.6

TABLE A21. EFFECT OF STORAGE ON BACTERIAL CONTENT OF OYSTERS FROM AREA M

Storage time, days	Storage temperature, °C	Coliform MPN/100 ml	E. coli MPN/100 ml	Plate count per ml	pH
Lot M1 - Shell oysters dug at Petit Pass-St. Joe 3-27-57, delivered to L. D. Gollott, Miss. 96, stored at room temperature.					
0		35,000		94,000	6.15
3/4	21-30	>160,000		380,000	6.38
1-1/3	21-30	92,000		200,000	6.48
2	21-22	>1,600,000		1,100,000	6.48
4	18-23	>1,600,000		TNC	6.47
6	18-19	>1,600,000		15,000,000	6.53
Lot M2 - Shell oysters same as lot M1, stored in refrigerator.					
0		35,000		94,000	6.70
3/4	2-8	35,000		59,000	6.76
4	2-9	23,000		170,000	6.95
8	2-10	540,000		150,000	6.78
Lot M3 - Shell oysters dug at Three Mile Reef 3-31-57, delivered to Fournier & Son, Miss. 63, 4-2-57, stored at room temperature.					
0		13,000		2,000	6.19
1/3	23-29	2,800		5,000	6.38
1-1/3	21-30	7,900		6,000	6.51
3	18-23	170,000		140,000	6.50
5	18-19	>1,600,000		430,000	6.28
Lot M4 - Shell oysters same as lot M3, stored in refrigerator.					
0		13,000		2,000	
3	2-12	13,000		2,000	6.75
7	3-9	4,600		7,000	6.72
Lot M5 - Shucked oysters dug 3-27-57, shucked by L. D. Gollott, Miss. 96, 4-1-57, stored at room temperature.					
0		4,900		61,000	
3/4	21-30	>160,000		TNC	5.67
1-1/3	21-30	>160,000		TNC	5.70
2	21-22	>1,600,000		TNC	4.98
Lot M6 - Shucked oysters same as lot M5, stored in refrigerator.					
0		4,900		61,000	
3/4	4	26,000		70,000	6.11
2	3-11	33,000		76,000	6.05
3	2-10	46,000		840,000	6.18
4	2-12	79,000		150,000	6.27
5	4-6	130,000		120,000	5.88
7	3-10	130,000		67,000	6.15
8	3-9	78,000		190,000	5.97
Lot M7 - Shucked oysters dug 3-31-57, shucked by Fournier & Son, Miss. 63, 4-2-57, stored at room temperature.					
0	23-29	160,000		43,000	6.10
1/3	23-29	>160,000		420,000	5.91
1-1/3	21-30	>1,600,000		TNC	5.65
2	21-22	>16,000,000		200,000,000	4.92
Lot M8 - Shucked oysters same as lot M7, stored in refrigerator.					
0		160,000		43,000	
1-1/3	3-11	79,000		47,000	6.08
2	2-10	350,000		49,000	6.14
3	2-12	350,000		52,000	6.10
4	4-6	130,000		56,000	5.98
6	3-10	170,000		100,000	6.02
7	3-9	240,000		160,000	6.01

TABLE A21. (continued) EFFECT OF STORAGE ON BACTERIAL CONTENT OF OYSTERS FROM AREA M

Storage time, days	Storage temperature, °C	Coliform MPN/100 ml	E. coli MPN/100 ml	Plate count per ml	pH
Lot M9 - Shucked oysters dredged at St. Joe Reef 11-4-57, delivered to C.C.Co., Miss. 13, 11-5-57, shucked 11-6-57, stored in refrigerator.					
Shellstock	40	>160,000		250,000	
0		>160,000		600,000	
2	7	280,000		360,000	6.28
6	0	170,000		250,000	6.12
9	2.5	22,000		210,000	6.0
17	2	49,000		480,000	5.75
20	4	130,000		300,000	5.75
Lot M10 - Shucked oysters dredged at Petit Pass Reef 11-19-57, delivered to C.C.Co., Miss. 13, 11-20-57, shucked 11-21-57, stored in refrigerator.					
Shellstock		130,000		330,000	6.42
0		920,000		260,000	
2	-1	350,000		380,000	6.29
5	3	350,000		310,000	6.08
8	3	350,000		360,000	5.9
Lot M11 - Shucked oysters (experimental lot) dredged at St. Joe Channel Reef 1-14-58, delivered to C.C.Co., Miss. 13, 1-14-58, shucked 1-15-58, stored in refrigerator.					
Shellstock		3,300	20	3,200	6.81
0		3,100	<18	4,300	7.0
2	0-4	13,000	60	14,000	6.31
6	2-7	2,400,000	18	1,000,000	6.30
8		1,100,000		100,000	5.8
Lot M12 - Shell oysters dredged at St. Joe Channel Reef 12-10-57, shucked 12-11-57 by Biloxi Seafood, Miss. 76, stored in refrigerator.					
Shellstock		35,000	78	7,900	6.9
0		35,000	180	44,000	6.5
3	2	23,000	<180	460,000	6.5
5	7	920,000	<180	23,000	6.1
7	7	1,400,000		7,600,000	6.0
11	7	24,000,000	78	34,000,000	5.5
Lot M13 - Shell oysters dredged at Three Mile Reef 2-8-58, shucked 2-13-58 by L. D. Gollott, Miss. 96, stored in refrigerator.					
Shellstock		160,000		16,000	
0		35,000	18	96,000	6.35
2	6	7,000	18	480,000	6.4
4	5	110,000		160,000	6.1
6	6	210,000		990,000	6.3
Lot M14 - Shell oysters dredged at Three Mile Reef 3-16-58, shucked 3-19-58 by L. D. Gollott, Miss. 96, stored in refrigerator.					
Shellstock		35,000		11,000	7.1
0		>160,000	330	40,000	6.9
2	7	33,000		34,000	6.4
7	5	1,600,000		TNC	5.9
15	8	4,800,000	20		5.2
Lot M15 - Shell oysters dredged at Morgan Harbor 3-27-58, shucked 4-3-58 by L. D. Gollott, Miss. 96, stored in refrigerator.					
Shellstock		>160,000	78	63,000	6.4
0		>160,000	20	130,000	6.6
2	6	>1,600,000		28,000	6.4
5	7	>160,000	68	1,200,000	6.0
8	8	14,000,000		6,700,000	5.2

TABLE A21. (continued) EFFECT OF STORAGE ON BACTERIAL CONTENT OF OYSTERS FROM AREA M

Storage time, days	Storage temperature, °C	Coliform MPN/100 ml	E. coli MPN/100 ml	Plate count per ml	pH
Lot M16 - Shell oysters dredged at American Bay 2-6-58, shucked 2-13-58 by Suarez Seafood, Miss. 129, stored in refrigerator.					
Shellstock		3,300		6,000	
0		>160,000	45	190,000	6.4
2	6	79,000		160,000	6.3
4	5	230,000	45	100,000	6.4
6	6	230,000		760,000	5.9
Lot M17 - Shell oysters dredged at Black Bay 3-12-58, shucked 3-19-58 by Suarez Seafood, Miss. 129, stored in refrigerator.					
Shellstock		17,000		TNC	6.65
0		22,000	790	TNC	6.45
15	8	11,000,000	220		4.95

TABLE A22. EFFECT OF STORAGE ON BACTERIAL CONTENT OF OYSTERS FROM AREA P

Storage time, days	Storage temperature, °C	Coliform MPN/100 ml	E. coli MPN/100 ml	Plate count per ml	pH
Lot P1 - Shucked oysters tonged at Pass Christian Reef 11-5-57, shucked 11-6-57 by Bennetts Seafood, Miss. 146, stored in refrigerator.					
Shellstock		2,300	93	1,800	6.72
0		3,300		4,700	6.57
2	5.5	540,000		400,000	6.12
6	0	490,000	<1,800	670,000	5.95
9	2.5	330,000		760,000	6.19
17	3	140,000		4,200,000	5.80
20	4	540,000		21,000,000	5.70
Lot P2 - Shucked oysters tonged at Pass Christian Reef 11-20-57, shucked 11-21-57 by Bennetts Seafoot, Miss. 146, stored in refrigerator.					
Shellstock		4,900	490	4,900	6.90
0		13,000	490	55,000	6.71
2	1	23,000	1,100	120,000	6.32
5	3	17,000		170,000	6.10
8	3	46,000	1,700	200,000	6.08
Lot P3 - Shucked oysters tonged at Pass Christian Reef 1-14-58, shucked 1-15-58 by Bennetts Seafood, Miss. 146, stored in refrigerator.					
Shellstock		3,300	74	3,800	6.70
0		7,000	78	4,000	6.47
2	0-3	92,000	37	8,300	6.28
6	2-7	9,200,000	78	540,000	6.30
8	2-7	3,500,000		490,000	5.80
Lot P4 - Shucked oysters tonged at Pass Christian Reef 4-2-58, shucked 4-3-58 by Bennetts Seafood, Miss. 146, stored in refrigerator.					
Shellstock		17,000	110	14,000	7.25
0		28,000	790	15,000	6.70
2	7	17,000		35,000	6.10
5	7	470,000	1,300	410,000	5.90
8	8	13,000,000		2,200,000	5.20
Lot P5 - Shell oysters tonged at Pass Christian Reef 12-10-57, shucked 12-11-57 by Kimball Seafood, Miss. 114, stored in refrigerator.					
Shellstock		7,900	130	4,700	6.9
0		3,300	78	25,000	6.4
3	3	4,900		42,000	6.1
5	8	49,000		88,000	6.1
7	7	350,000		980,000	5.85
11	7	92,000,000	110	12,000,000	5.6

TABLE A23. COLIFORM, SALMONELLA, E. COLI MPN'S, AND STANDARD PLATE COUNTS IN STORED SHUCKED OYSTERS

(Experiment 1)

Storage, days	MPN per 100 grams			Standard plate count per gram
	Salmonella	Coliform	E. Coli	
	Storage at 4° - 7°C			
0	33,000	3,100	40	2,500
1	7,900	7,000		2,400
2	33,000	33,000	20	8,600
3	3,300	310,000	45	74,000
6	11,000	2,200,000	< 18	1,600,000
7	23,000	4,900,000	18	1,800,000
8	3,300	4,900,000	< 18	2,900,000
9	4,900	7,900,000	< 18	3,800,000
10	4,300	24,000,000	< 18	4,200,000
	Storage at 20°C			
0	33,000	3,100	40	2,500
1/3	17,000	33,000	20	6,300
1	1,600,000	23,000,000	3,500	3,900,000

TABLE A24. COLIFORM, SALMONELLA, E. COLI MPN'S, AND STANDARD PLATE COUNTS IN STORED SHUCKED OYSTERS

(Experiment 2)

Storage, days	MPN per 100 grams			Standard plate count per gram
	Salmonella	Coliform	E. Coli	
	Storage at 4° - 7°C			
0	3,300	17,000	220	6,500
1	11,000	33,000	78	60,000
2	4,900	130,000	20	100,000
4	3,300	2,400,000	78	1,300,000
5	3,500	13,000,000	78	7,000,000
6	3,300	2,300,000	40	10,000,000
8	330	7,900,000	45	13,000,000
	Storage at 20°C			
0	3,300	17,000	220	6,500
1/3	4,900	79,000	460	88,000
1	130,000	23,000,000	130,000	12,000,000

TABLE A25. COLIFORM, SALMONELLA, E. COLI MPN'S, AND STANDARD PLATE COUNTS IN STORED SHUCKED OYSTERS (Experiment 3)

Storage, days	MPN per 100 grams			Standard plate count per gram
	Salmonella	Coliform	E. Coli	
			Storage at 4° - 7°C	
0	4,900	23,000	1,300	11,000
1	7,900	33,000	330	60,000
3	7,000	230,000	5,400	400,000
5	2,800	330,000	1,400	1,900,000
6	7,900	3,300,000	140	5,300,000
7	3,100	35,000,000	310	990,000
			Storage at 20°C	
0	4,900	23,000	1,300	11,000
1/3	13,000	24,000,000	1,700	57,000
1	110,000	49,000,000	160,000	5,000,000

TABLE A26. COLIFORM, SALMONELLA, E. COLI MPN'S, AND STANDARD PLATE COUNTS IN STORED SHUCKED OYSTERS (Experiment 4)

Storage, days	MPN per 100 grams			Standard plate count per gram
	Salmonella	Coliform	E. Coli	
			Storage at 4° - 7°C	
0	4,900	23,000	490	13,000
1	11,000	110,000	790	110,000
2	2,200	230,000	2,400	4,600,000
4	4,600	3,500,000	790	5,800,000
6	2,200	13,000,000	1,300	16,000,000
7	4,600	17,000,000	210	3,500,000
8	9,500	49,000,000	130	5,100,000
			Storage at 20°C	
0	4,900	23,000	490	13,000
1/3	11,000	95,000	4,600	35,000
1	1,700,000	49,000,000	330,000	21,000,000

TABLE A27. COLIFORM, SALMONELLA, E. COLI MPN'S, AND STANDARD PLATE COUNTS IN STORED SHUCKED OYSTERS (Experiment 5)

Storage, days	MPN per 100 grams			Standard plate count per gram
	Salmonella	Coliform	E. Coli	
			Storage at 4° - 7°C	
0	4,900	1,300	130	4,600
1	13,000	17,000	220	5,800
2	7,000	33,000	140	8,500
3	2,800	79,000	490	140,000
4	13,000	330,000	490	710,000
5	4,600	490,000	74	690,000
6	4,600	310,000	490	700,000
7	1,700	950,000	490	1,400,000
8	4,900	3,300,000	790	2,600,000
9	3,300	3,300,000	330	790,000
			Storage at 20°C	
0	4,900	1,300	130	4,600
1/3	7,900	9,500	78	5,300
1	490,000	11,000,000	230,000	2,400,000

TABLE A28. COLIFORM, SALMONELLA, E. COLI MPN'S, AND STANDARD PLATE COUNTS IN STORED SHUCKED OYSTERS
(Experiment 6)

Storage, days	MPN per 100 grams			Standard plate count per gram
	Salmonella	Coliform	E. Coli	
	Storage at 4° - 7°C			
0	1,700	330,000	1,100	23,000
1	7,900	490,000	7,900	50,000
2	4,600	2,200,000	4,900	150,000
3	1,700	2,200,000	11,000	390,000
4	17,000	4,900,000	2,300	500,000
6	4,600	7,900,000	2,200	10,000,000
7	3,500	13,000,000	1,100	18,000,000
8	2,200	35,000,000	1,400	31,000,000
	Storage at 20°C			
0	1,700	330,000	1,100	23,000
1/3	7,900	3,300,000	4,700	110,000
1	240,000	1,300,000,000	13,000,000	27,000,000

TABLE A29. COLIFORM, SALMONELLA, E. COLI MPN'S, AND STANDARD PLATE COUNTS IN STORED SHUCKED OYSTERS
(Experiment 7)

Storage, days	MPN per 100 grams			Standard plate count per gram
	Salmonella	Coliform	E. Coli	
	Storage at 4° - 7°C			
0	4,600	49,000	< 18	8,800
1	33,000	49,000	< 18	43,000
2	33,000	330,000	< 18	220,000
3	11,000	790,000	< 18	290,000
6	4,900	11,000,000	< 18	920,000
7	3,300	7,900,000	< 18	2,600,000
8	7,900	4,900,000	< 18	2,600,000
9				
10				
	Storage at 20°C			
0	4,600	49,000	< 18	8,800
1/3	3,100	2,400,000	< 18	27,000
1	1,700,000	49,000,000	40	9,400,000